A longitudinal analysis of neonatal and infant diagnostic HIV-PCR uptake and associations during three sequential policy periods in Mitchell's Plain, Cape Town

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KLKEMM001

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DECLARATION

I, Emma Kalk, hereby declare that the work on which this dissertation is based is my original work (except where acknowledgements indicate otherwise) and that neither the whole work or any part thereof has been, is being or will be submitted for another degree at this or any other university. I further declare that the work was not published prior to my registration for the degree of Master of Public Health.

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ABSTRACT

Background: Despite technological and programmatic advances in the prevention of vertical transmission of HIV and early infant diagnosis (EID), paediatric HIV continues to have a significant impact on infant and child survival in low- and middle-income countries. Many EID programmes follow the WHO recommendation of initial infant HIV testing with a nucleic acid assay at 4-6 weeks old. In general this strategy has been poorly implemented with substantial attrition after birth such that, according to UNAIDS, only 51% of HIV-exposed infants received a virological test in the first two months of life in 2015. In addition, there is concern about the sensitivity of the nucleic acid assays at six weeks in the context of exposure to prolonged multidrug antiretroviral therapy as infant post-exposure prophylaxis, and in breast milk. HIV-PCR testing at birth has been promoted as a means of maximizing the number of infants who receive an HIV test as well as identifying *in utero*-infected infants in whom HIV infection may follow an aggressive course.

Evidence from pilot studies and modelling data was sufficiently compelling for the WHO to include a conditional recommendation for the addition of a birth HIV-PCR (either routine or targeted at high risk groups) to its EID algorithms in 2015. The Western Cape introduced targeted birth HIV testing for high risk infants in August 2014 and expanded this in line with the South African National Prevention of Mother-to-Child Transmission Guidelines, to include all HIV-exposed infants in December 2015.

Methods: Between 2013 and 2016 we conducted an implementation science project to iteratively assess the implementation and effectiveness of the vertical transmission prevention of HIV in a chain of referral facilities in Cape Town (i.e. from primary to tertiary care). The e-register provided a single longitudinal record for each woman (linked to her infant after birth) and enabled assessment of HIV testing and treatment from first antenatal visit through delivery to infant HIV testing.

Using a cohort of HIV-exposed live infants from this database, a protocol was designed (Section A: Protocol) to assess the implementation and outcome of effectively three different EID policy periods in the facility chain. i.e. an initial period of birth HIV-PCR at the

clinician's discretion if evidence of HIV infection; a period of targeted birth testing of high risk infants and lastly, of routine birth HIV-PCR for all HIV-exposed infants.

A critical review of the literature appraised published assessments of birth HIV testing programmes in low- and middle-income countries (Section B: Literature Review) with the aim of assessing *in utero* transmission rates, follow-up testing and transmission rates and the resources required for implementation. Studies that modelled the impact of birth HIV testing were specifically reviewed.

The manuscript (Section C: Manuscript) presented an analysis of the HIV-infected/exposed mother/infant dyads from the e-register of the Closing the Gaps study. Using this database adherence to guidelines in each period was assessed as well as the outcome of HIV-PCR at four delivery sites and the impact of the policies on return for follow-up EID.

Results: South Africa is the first country in sub-Saharan Africa to implement birth HIV testing and most of the studies in support of this strategy were generated here. There was limited literature which highlighted the need for further investigation into the implementation and effectiveness of such programmes. No prospective data addressed targeted birth testing and those reporting on more routine birth HIV-PCR demonstrated success in timeous diagnosis and treatment although significant additional project resources were required. The retrospective laboratory data indicated that receipt of a birth HIV-PCR reduced the likelihood for follow-up at later testing time-points. This is important as the modelling studies suggested that the clinical and financial benefits of adding birth testing to existing algorithms would be lost if follow-up was poor.

In the cohort of 2012 HIV-exposed infants in the study presented in the manuscript, the proportion who received birth testing increased with the progression of the EID policies but guideline implementation was poor, especially in primary care, with only 60% of infants being tested as recommended. The proportion of infants with positive HIV-PCR decreased as the pool of HIV-exposed infants undergoing testing expanded, being highest during the periods of targeted birth testing. In concurrence with the South African literature, receipt of a birth HIV-PCR decreased the likelihood of follow-up testing at 6-10 weeks. Among infants tested at 6-10 weeks old, the proportion who were positive for the first time at this time-

point increased with the introduction of routine birth testing for all HIV-exposed infants, emphasizing the importance of the follow-up EID time-points.

Conclusion: Virological testing at birth effectively increased the number of HIV-exposed infants who received an HIV test and was effective in identifying *in utero* infection in high risk infants (who could start treatment early with the attendant benefits.) The Western Cape EID policies were incompletely implemented in the study facilities over this time with many infants not being tested as indicated. Birth HIV-PCR decreased follow-up testing, an unintended consequence of serious concern. Adherence to the provincial and national guidelines needs to be re-enforced at delivery sites and at the primary care facilities where follow-up EID occurs.

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LIST OF ABBREVIATIONS

ANC	Antenatal Care
ART	Antiretroviral Therapy
ARV	Antiretrovirals
AZT	Zidovudine
CEPAC	Cost-Effectiveness of Preventing AIDS Complications
DBS	Dried Blood Spots
EID	Early Infant Diagnosis
GSH	Groote Schuur Hospital
HIV	Human Immunodeficiency Virus
LTFU	Lost to Follow-up
LMIC	Low and Middle Income Countries
MMH	Mowbray Maternity Hospital
MOU	Midwife Obstetric Unit
MPMOU	Mitchell's Plain MOU
MPDH	Mitchell's Plain District Hospital
MTCT	Mother-to-Child Transmission
NAT	Nucleic Acid Test
NHLS	National Health Laboratory Services
NVP	Nevirapine
PCR	Polymerase Chain Reaction
PGWC	Provincial Government of the Western Cape
PMTCT	Prevention of Mother-to-Child Transmission
POC	Point of Care
SA	South Africa
UNAIDS	The Joint United Nations Programme on HIV and AIDS
VL	Viral Load
VTP	Vertical Transmission Prevention
WHO	World Health Organization

PART A. RESEARCH PROTOCOL

SYNOPSIS

Title: A longitudinal analysis of neonatal and infant diagnostic HIV PCR uptake and associations during three sequential policy periods in Mitchell's Plain, Cape Town

Background and Rationale: WHO guidelines recommend nucleic acid testing (HIV DNA-PCR or equivalent) of HIV-exposed infants between 4-6 weeks of age. At this time-point most *in utero* and intra-partum HIV infections can be detected; in addition it is programmatically convenient as it coincides with a routine immunization visit. There is a subsequent visit at 10 weeks old at which the results can be returned. However, there is significant attrition before 4-6 weeks and the majority of HIV-exposed infants are not tested by two months old; and, operationally antiretroviral therapy (ART) initiation may be delayed in HIV-infected infants with consequences for morbidity and mortality. In 2015 the WHO added a conditional recommendation for diagnostic HIV PCR testing of HIV-exposed infants at birth to identify *in utero* infected neonates *in addition to* testing at a later time-point. Prior to the WHO recommendation, in 2014 the Western Cape Province added birth HIV PCR testing for neonates at high risk of vertical infection to the early infant diagnosis (EID) algorithm; and in 2015 routine birth testing was introduced for *all* HIV-exposed infants. The implementation and impact of these guideline changes are unknown.

Purpose and Objectives: The purpose of this study is to examine the uptake of HIV PCR testing under three different EID policies (i.e. six weeks only; birth for high risk infants and six weeks; and birth for all exposed infants and 10 weeks) in the Western Cape, and the diagnostic yield at each time-point. Findings will be placed in the context of the evolving local prevention of vertical transmission of HIV recommendations and international clinical and programmatic guidelines.

Methods: The proposed project is a sub-analysis of data collected from the larger Closing the Gaps study, an implementation science project which developed an electronic system allowing iterative assessment of the vertical transmission prevention of HIV programme at a facility and individual level. We will include live infants born to HIV-infected women who

received antenatal care and/or delivered at Mitchell's Plain Midwife Obstetric Unit and its referral centres in Cape Town between February 2014 and June 2016.

Descriptive statistics will be used to describe HIV PCR testing uptake and how this changed as the EID programme evolved. Logistic regression will be used to determine predictors of testing uptake in the context of the different guidelines.

Risks and Benefits: The major risk of this sub-study mirrors that of the parent project: breach of confidentiality as study staff (EK) will have access to individually identifiable maternal and infant HIV-related data.

Risks, Potential Benefits and Protection Against Risks: Inclusion of identifiers in the database is necessary initially so that women and their infants can be linked and the data cleaned (which may require return to the original sources). Identifiers will be removed from all data as soon as linkage and cleaning are complete. Only staff directly involved (EK) will have access to any individually identifiable data. EK has undergone the NIH Human Subjects Protection training, has completed Good Clinical Practice training in the past and, as a registered medical practitioner with the HPCSA is bound by professional medical standards. All electronic data will be password-protected and stored on a secure server housed in the CIDER offices at the UCT, with user-level access control.

The risk of breach of confidentiality is small and the study is considered low-risk.

There are no direct benefits to the participants. Indirectly, the analysis will allow measurement of the uptake of HIV PCR testing and HIV diagnosis rates during three different EID strategies as they were implemented in the Mitchell's Plain over the study period. Such evaluation is important locally and internationally; South Africa is one of the few low or middle income countries where birth HIV testing has been introduced and such assessments are lacking in the region.

PROTOCOL

A longitudinal analysis of neonatal and infant diagnostic HIV PCR uptake and associations during three sequential policy periods in Mitchell's Plain, Cape Town

1. Background

Despite advances in prevention of vertical transmission of HIV strategies and treatment, paediatric HIV infection remains a global challenge. Without treatment, HIV infection is rapidly progressive in infants and young children with 50% mortality within the first two years of life (1). In South Africa, mortality of vertically infected untreated infants peaks at 2-3 months of age (2). Evidence from observational studies and randomized controlled trials (most notably the Children with HIV Early Antiretroviral Therapy [CHER] trial (3)) indicates that early treatment of HIV infected infants with antiretroviral therapy (ART) reduces mortality and abrogates early and long-term complications (4, 5). In the CHER study ART initiated before seven weeks of age was associated a 76% decreased mortality and a 75% drop in HIV disease progression (3). More recent data suggest that early treatment (within hours to days of birth) can limit viral replication and reduce immune suppression and physiological damage (6).

Early ART initiation, with its attendant benefits, depends on early diagnosis of HIV infection. Diagnostic technological advances have now pushed back diagnostic possibility to within hours of birth.

Since 2007 the World Health Organization (WHO) recommendation for early infant diagnosis (EID) has been an initial nucleic acid test (HIV PCR) at six weeks old (7, 8). Early studies indicated that testing at this time point would detect the majority of *in utero* and intra-partum infections and operationally it coincides with the routine six week extended programme on immunization (EPI) vaccination visit recommended by the WHO (9, 10). Since the above studies were conducted, prevention of vertical transmission of HIV (VTP) programmes have expanded in sophistication and scope with prolonged and more effective drug treatment and more sensitive and efficient diagnostic assays (11). In South Africa the majority of HIV-infected pregnant women who attend antenatal care are initiated on ART (93% in 2015/2016(12)) and intra-partum transmission (which accounted for upward of 50%

of infant HIV infections in untreated women (13)) has been reduced. As a result a larger proportion of infant infections are due to *in utero* (i.e. before starting ART) and post-partum transmission.

These advances, together with the clinical benefits of very early ART initiation, have called into question the appropriateness of the six week time point for initial diagnostic HIV testing. Prolonged infant post-exposure prophylaxis and maternal ART now routine in many VTP programmes in low and middle income countries (LMIC) and the sensitivity and specificity of the diagnostic HIV PCR at six weeks in this context is unclear.

In sub-Saharan Africa, there is significant attrition from VTP care with the majority of HIV exposed infants failing to receive the six week HIV PCR (14). This, coupled with laboratory delays and difficulties communicating results to sites, resulted in only an estimated 50% of HIV-infected infants initiating ART in 2016 (15). Even when programme timelines are observed, infants testing HIV positive at six weeks old may only initiate treatment at 14 weeks, beyond the recommended seven weeks of CHER and the 2-3 month peak in HIV-related mortality (2, 3, 16). Evidence from Johannesburg suggested that 76% of infant HIV infections diagnosed at six weeks could be detected at birth, potentially allowing for more rapid ART initiation. In settings with high rates of institutional deliveries, such as South Africa, birth testing would achieve higher coverage than the six week test (11) and, models suggest, save the same number of life years (17). Furthermore, the potential clinical benefits of early treatment (hours to days) in preserving immune function and restricting viral reservoirs support the move to a more timeous diagnosis (6).

Given these issues, the WHO included a conditional recommendation in the 2015 VTP guidelines for an HIV PCR around birth (0-2 days) *in addition to* testing at 4-6 weeks (8). The rationale being that testing soon after birth would identify infants infected *in utero* in whom HIV disease is severe and rapidly progressive allowing timeous initiation of ART. In settings were the majority of births occur in health facilities, exposed neonates could be tested before discharge and resources focused on drawing positive infants into care. Concerns about the sensitivity of the PCR assays in the presence of prolonged infant prophylaxis (for example, daily nevirapine [NVP] for at least six weeks) would not apply (16).

Early Infant Diagnosis in the Western Cape (Figure 1)

In line with the South African National Guidelines, the 2013 Western Cape *Prevention of Mother-to-Child Transmission of HIV (PMTCT) Clinical Guidelines Update* recommended routine HIV PCR of all HIV exposed infants at six weeks old (18). Provision was made for a diagnostic PCR at birth at the attending clinician's discretion (e.g. in situations of suspected nevirapine [NVP] resistance or clinical symptoms). In July 2014 an additional test at birth was added for those infants who met high risk criteria for vertical infection (19)(Table 1.)

The South African *National Consolidated Guidelines for the Prevention of Mother-to-Child Transmission of HIV (PMTCT) and the Management of HIV in Children, Adolescents and Adults* introduced routine HIV diagnostic testing for all HIV-exposed infants at birth in 2015 with repeat testing at 10 weeks to identify peri-partum and early postnatal infections (20). This was implemented in the Western Cape in November 2015 (21).

Thus, over the projected course of this project, the Western Cape EID recommendations were updated twice allowing us to assess uptake and results of HIV PCR testing under three different EID strategies (Figure 1).

Period 1: Routine PCR at six weeks old; birth PCR at clinician's discretion.

Period 2: Birth PCR for infants meeting high risk criteria for vertical transmission of HIV (Table 1) with routine diagnostic testing at six weeks old.

Period 3: Routine birth PCR for all HIV-exposed infants with follow-up PCR at 10 weeks old.

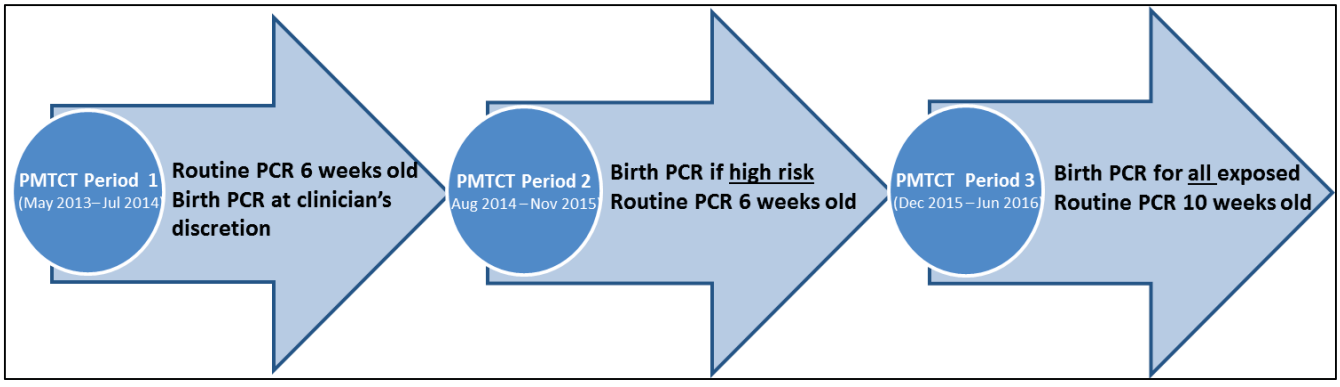

Figure A.1. Evolving Western Cape Prevention of Mother-to-Child Transmission of HIV guidelines giving three policy periods between 2013 and 2016.

MATERNAL FACTORS	INFANT FACTORS
Viral load >1000 copies/ml after 28 weeks gestation	Born before 37 weeks gestational age
ART < 12 weeks of before delivery	Birthweight < 2500g regardless of gestation
Defaulted ART for ≥ 1 month during pregnancy	Abandoned babies and orphans
Newly diagnosed with HIV > 28 weeks gestation including during labour/immediately postpartum	Symptomatic
Likely NNRTI resistance (on 2^{nd}/3^{rd} line ART during pregnancy	
Diagnosed with TB or syphilis during pregnancy	
Clinical signs of chorioamnionitis	

Table A.1. Criteria for identifying HIV exposed infants at high risk of vertical infection (birth PCR indicated in terms of the 2014 Western Cape Guidelines (22)) ART – antiretroviral therapy; NNRTI – non-nucleotide reverse transcriptase inhibitor; TB – tuberculosis disease

2. Justification

There has been rapid, if patchy, uptake of birth HIV PCR testing in South Africa since 2013 but limited assessment of its impact (12). Review of routine programme implementation was identified as a research priority by the National Institute of Allergy and Infectious Diseases workshop on "HIV Birth Testing and Linkage to Care for HIV Infected Infants" in 2016, the aim of the which was to specifically evaluate birth testing (23). The project offers the opportunity to measure and compare the uptake and impact of birth HIV PCR testing at different levels of care (primary, secondary and tertiary) and under three different EID policies.

In addition, it is critical that infants with a negative birth HIV test result return for repeat testing (at 10 weeks old according to current local recommendations) to detect intra-partum and early post-natal infections, and that ART is initiated rapidly. There are concerns that receipt of a negative birth HIV PCR result will reduce return for follow-up testing.

This study will allow us to assess adherence to provincial guidelines, the usefulness of HIV PCR testing at birth at different delivery sites and the impact on presentation for follow-up HIV PCR testing in a single referral system under the three different EID strategies described above.

3. Hypotheses

Hypothesis 1: Birth HIV PCR testing has improved the overall proportion of HIV-exposed infants tested as part of EID.

Hypothesis 2: The VTP programme in the Western Cape is effective and incidents of vertical transmission are typified by certain characteristics (risk factors).

Hypothesis 3: Receipt of a negative birth HIV PCR result decreases up-take of follow-up HIV PCR testing at 10 weeks and beyond.

4. Aim

The overall aim is to describe the uptake of HIV PCR testing (EID) by HIV-infected women who attended a single primary care antenatal site under three sequential EID policy periods and to determine associations between timing of HIV PCR testing and clinical and demographic characteristics in each of these.

5. Objectives:

1. To described the implementation of the Western Cape Provincial EID guidelines including the uptake of infant HIV PCR testing (birth and subsequently) by HIV-infected women during three sequential EID policy periods.

2. To determine the clinical and demographic associations that impact on the EID uptake at different time-points (i.e. birth and 6-10 weeks).

3. To determine the proportion of positive HIV PCR results and describe clinical and demographic characteristics of HIV-infected infants.

6. Methods

This project will form a sub-analysis of the larger, Closing the Gaps (CTG) study (Appendix 1). CTG was an NIH-funded implementation science project in the form of a prospective cohort of pregnant women and their infants comparing the VTP coverage and effectiveness before and after the implementation of an active VTP surveillance system – an electronic or e-register. Using the existing medical records systems, CTG utilized routinely collected clinical data from existing Provincial data sources, namely the paper-based obstetric registers in use

at Mitchell's Plain Midwife Obstetric Unit (MPMOU) and the referral hospitals. Data elements from the antenatal HIV testing registers, the labour ward HIV PMTCT registers, and delivery (birth) registers were consolidated into the e-register. HIV-associated laboratory data and ART prescription data were included.

6.1 Study design

Prospective cohort study

6.2 Population and sampling

This study will utilize data from the live infants born to HIV-infected women in the CTG cohort. The CTG database comprised all pregnant women who presented for antenatal care at the MPMOU in Cape Town and who delivered at MPMOU or any of its referral centres (Mitchell's Plain District, Mowbray Maternity and Groote Schuur Hospitals) between February 2014 and June 2016. Pregnant women (regardless of HIV status) were enrolled prospectively as they presented at the facility for their first antenatal visit or in labour if they had not received any antenatal care. There were no exclusion criteria.

Clinical and socio-demographic characteristics

Mitchell's Plain Community Health Centre, of which MPMOU is a part, has catchment area of approximately 1.2 million people covering the geographic areas of Mitchell's Plain (an urban lower to middle income historically "Coloured" township with formal housing) and the neighboring suburbs of Phillipi and Crossroads housing poorer, predominantly black African communities. In these areas there is a mix of formal and informal housing. Unemployment was 24.2% in 2015 (24).

Women received antenatal care at MPMOU or nearby basic antenatal care (BANC) clinics. Uncomplicated vaginal births were managed by midwives at the MOU. Approximately half of all women who attend the facility antenatally were referred to hospital either during pregnancy or peripartum if deemed high risk or requiring advanced care. MPMOU refers to Mitchell's Plain District Hospital (level 1 with operating theatres), Mowbray Maternity Hospital (level 2 with neonatal ICU facilities), and Groote Schuur Hospital (level 3 with adult and neonatal ICU facilities).

6.3 Sample size

Approximately 7000 women attend antenatal care (book) at MPMOU per year about half of whom deliver at the facility (the remainder being referred to hospital). HIV prevalence at delivery during the study period was 14% and we would expect approximately 2000 HIV-exposed infants over two years.

6.4 Research procedures and data collection methods

This analysis will use data collected as part of the CTG study. CTG enrolled women between February 2014 and June 2016. Delivery information was collected through the end of 2016. Women identified as HIV-infected will be extracted from the larger database of the entire CTG cohort and pregnancy losses excluded, giving a cohort of HIV-exposed live-born infants. The maternal data will be linked via infant folder number with the corresponding infant profiles to allow analysis of maternal and delivery information and infant anthropometry and HIV test results. The Western Cape Province has introduced a system of unique patient identifiers: all clients of the provincial health services use the same unique folder number at every facility. Blood and other laboratory tests are also requested with this number. Thus all HIV-relevant laboratory investigations (e.g. maternal viral load and infant HIV PCR; see below) can be confirmed, and mothers and their infants linked.

All data will be de-identified before analysis.

Exposures

The antenatal HIV prevalence in the Western Cape at the time of data collection was 18.7% (25). The Western Cape Provincial Government PMTCT clinical guidelines underwent two updates during the study period (Figure 1). WHO Option B+ was introduced in the Province in 2013 and this was the overall strategy throughout the study. WHO Option B+ recommends initiating lifelong ART (i.e. treatment with three antiretroviral agents from two different classes) in all HIV-infected pregnant women regardless of clinical or immunological stage; it replaced less aggressive strategies which prescribed single or dual VTP prophylaxis for women with higher CD4 cell counts. Maternal first-line ART consisted of tenofovir (TDF), emtricitabine (FTC) and efavirenz (EFV). HIV-exposed infants received six weeks of daily NVP if the mother had received more than eight weeks of ART before delivery or had elected to

exclusively formula feed. Breastfed infants whose mother's had received less than eight weeks ART antenatally were given daily NVP for 12 weeks. For infants at high risk of transmission zidovudine (AZT) was added. In 2015 these were amended: those neonates at low risk of transmission at birth received six weeks of NVP regardless of feeding choice. For high risk infants NVP was extended to 12 weeks and AZT added for the first six weeks(26).

HIV PCR testing schedules are described in Figure 1 above. The PMTCT and EID algorithms for each period are detailed in Appendix 2.

Well baby clinics and infant HIV PCR testing usually occurred at sites separate from the maternity facilities and in some cases were managed by a different arm of local government. At these sites, clinic staff ascertained HIV-exposure status (and hence the need for an HIV PCR) either from the caregiver or from the client-held Road-to-Health book given to all infants born in South Africa which serves as a record of birth, immunizations, HIV exposure and VTP, and growth.

Laboratory measurements

Relevant laboratory results from the NHLS (i.e. maternal CD4 count and viral load measurements, and infant HIV PCR results) were abstracted from the electronic NHLS platforms and entered into the e-register. The NHLS provides a quality controlled and validated laboratory service for all government health facilities. In order to ensure that all available results are collected, an output of relevant results for CTG participants will be requested from the Provincial Health Data Centre (PHDC) which acts as a repository for all electronic medical systems including the NHLS.

Linking maternal and infant data

At all primary health care facilities in the Western Cape Province, including MPMOU, appointments and key delivery data are entered on the Primary Health Care Information System (PHCIS). After delivery and before the mother-infant pair is discharged, selected delivery data are entered in the Maternity Module of PHCIS and an infant folder number is automatically generated. This links the mother-infant pair and is recorded in the maternity record and infant Road-to-Health book. The equivalent hospital system, Clinicom, generates a linked infant folder number in a similar process. These infant numbers were entered into

the maternal profile in the e-register of the CTG cohort allowing linkage of maternal and infant data.

PHCIS data entry at MPMOU was done by the midwives and compliance was poor at the start of the project, resulting in missing infant numbers and incomplete linkage. This improved within four months of the study and the majority of live-born neonates were appropriately identified. The sample will be limited to those mother-infant pairs whom we were able to link.

Maternal entries containing relevant results and delivery information will be linked with infant entries recording birth anthropometry and HIV-related blood results.

Endpoints/variables

Using the cohort of HIV-exposed live-born infants with an unique identifier, evidence of all HIV PCR tests per infant in defined time periods and the results of these will comprise the main outcome variables. Participants will be categorized as follows: no HIV PCR before 11 weeks old, birth HIV PCR only, birth HIV PCR and follow-up PCR (6-10weeks), HIV PCR at 6-10 weeks only.

Associations of testing at the different time-points with maternal and infant characteristics (including the high risk criteria) will be assessed (Table 2).

6.5 Data management

Closing the Gaps was an implementation science project and efforts were made to use existing facility systems. Routinely collected data was entered directly from the clinical records into eKapa, the networked electronic medical records system in place at MPMOU. At the time of the study, eKapa was in routine use for the capture of HIV-related patient information in HIV-infected patients but did not include any obstetric parameters. We augmented the routine system to include HIV negative women as well as obstetric data on all women which was entered into a Study Module. Individual records were created for infants which were subsequently linked to the corresponding maternal records via the infant folder numbers as described. Exports of data on enrolled subjects were requested for cleaning and then analysis.

Using the export of raw data from the CTG cohort I will restrict the sample to pregnant women identified as HIV-infected. From these women I will extract those who had the live births (excluding pregnancy loss [stillbirth, miscarriage, termination of pregnancy, ectopic pregnancy and molar pregnancy], false pregnancies and those for whom no outcome can be found). I will merge these records with the corresponding infant records using the infant identifier resulting in a database of HIV-exposed live born infants, and including the variables outlined below. If no infant number can be established, the mother-infant pair will be excluded.

Once these linkages are complete, I will allocate a unique study identifier to each participant and data analysis will be conducted on an anonymized dataset.

Variable	Description	Detail
PMTCT policy period	Categorical	(Period 1,2 or 3)
High risk factor*	Binary (present/absent)	
Delivery at hospital	Binary (yes/no)	Versus primary care facility (i.e. MPMOU)
Mode of delivery	Categorical	Vaginal/Assisted/Caesarian
Maternal factors		
Age (years)	Numerical - continuous	
Primigravida	Binary (yes/no)	Versus second or subsequent pregnancy
Viral load >1000 copies/ml after 28 weeks gestation	Binary (yes/no/not available)	
ART < 12 weeks of before delivery	Binary (yes/no/not available)	
Defaulted ART for ≥ 1 month during pregnancy	Binary (yes/no/not available)	
Newly diagnosed with HIV > 28 weeks gestation including during labour/immediately postpartum	Binary (yes/no)	
No antenatal care	Binary (yes/no/not available)	
Neonatal factors		
Premature birth : <37 weeks gestation†	Binary (yes/no)	
LBW <2500g regardless of gestation	Binary (yes/no)	

Table A.2. Variable list: baseline characteristics including high risk factors for vertical transmission of HIV

*High risk factors: Viral load >1000 copies/ml after 28 weeks gestation; ART < 12 weeks of before delivery; Newly diagnosed with HIV > 28 weeks gestation including during labour/immediately postpartum; No antenatal care; Premature birth; LBW

†Gestational age as recorded in the labour ward delivery registers at the site. This is usually based on antenatal assessment as Ballard Scores are not routinely performed.

PMTCT – Prevention of Mother-to-Child Transmission; MPMOU – Mitchell's Plain Midwife Obstetric unit; ART – Antiretroviral Therapy; LBW – Low Birth Weight

6.6 Analysis

Data will be analyzed using STATA version 15.0 (Stata Corporation, College Station, Texas, USA).

Continuous variables will be summarized using means and confidence intervals (CI) or medians and interquartile ranges (IQR) for normally and non-normally distributed variables respectively. Categorical variables will be described using proportions and frequency tables will be used to compare proportions.

Significance will be tested using a two-sample t-test or Wilcoxon rank-sum test depending on the distribution for numerical data and the $\chi 2$ test or Fishers Exact test for categorical data.

Logistic regression will be used to identify the predictors of PCR timing. Multivariate models will be fitted including known or suspected risk factors for the primary outcomes.

A significance level of 0.05 will be used for all statistical tests.

7. Ethical considerations

The Closing the Gaps study was approved by the Human Research Ethics Committee of the University of Cape Town (HREC: 145/2013; Appendix 3) and the Provincial Government of the Western Cape Department of Health Research (RP063/2013; Appendix 3). This protocol comprises the application for the sub-analyses described above.

The CTG electronic register used patient folder number (maternal and infant), name, birth dates and delivery dates as linkage fields when consolidating the registers and laboratory results and when linking mother and infant.

7.1 Informed consent

There was no recruitment for the electronic register and a waiver of informed consent was granted for this part of the project since only data that was already part of routine care by the health services were collected. Data were entered on the existing provincial medical records platform by a single trained data clerk who was bound by professional and ethical

considerations when she was granted access to the system. She completed the NIH Protection of Human Subjects on-line training.

7.2 Description of risks and benefits

The major risk of this study is breach of confidentiality as I will have access to individually identifiable maternal and infant health information including HIV data. The study is considered to be low risk with the potential for loss of confidentiality during the process of data cleaning and linkage.

7.3 Protection against risks

Since every effort will be taken to ensure confidentiality, the small risk of it being breached can be considered to be acceptable. To minimize the risk of breaching confidentiality, all electronic data will be stored in a password-protected database on a secure server housed in the CIDER offices. As soon as linkage and data cleaning are complete all identifiers will be removed, prior to analysis. A unique study identifier will be allocated to each participant and data analysis will be conducted on an anonymized dataset.

Any data transfer will be done securely with password protection via the UCT data transfer web-platforms, the passwords being sent separately and by a different route (e.g. text message to mobile phone) to the databases.

7.4 Potential benefits

There is no direct benefit to participants as this will be a retrospective analysis of routinely collected clinical data.

Indirect benefit will stem from the analysis of the database which may identify gaps or challenges in the implementation of the Provincial VTP guidelines. In addition, variables associated with a high risk of vertical transmission or failure to implement guidelines may be ascertained, allowing for the antenatal identification of vulnerable women and infants who may require additional services and support. Ideally, such systems could be incorporated into future VTP programmes.

7.5 Reporting and implementation

The results of this sub-study will be submitted to a peer-review publication and presented at appropriate conferences. A report will be submitted to the HAST directorate at the KMP SS and the facility and operational managers at the MPMOU, MPDH, MMH and GSH for dissemination to staff.

8. Logistics

Timetable

Task	Duration
Merge, clean and check datasets	9 months
Analysis	2 months
Preparation draft manuscript	2 months
Preparation final manuscript and submission for publication	3 months

Budget

This analysis and write-up will form part of the requirements for my MPH and no budget is required.

9. Appendices

Appendix 1: *Closing the Gaps in PMTCT Programme Coverage, Early Infant Diagnosis and Treatment (CTG)*

Appendix 2: Western Cape Vertical Transmission Prevention of HIV and Early Infant Diagnosis Algorithms

a) PMTCT Clinical Guidelines Update May 2013

b) PMTCT Clinical Guidelines Update June 2014

c) Western Cape Consolidated Guidelines for HIV Treatment: Prevention of Mother-to-Child Transmission of HIV (PMTCT), Children, Adolescents and Adults. 2015 (Amended version)

Appendix 3: UCT Human Research Ethics Committee approval (145/2013); PGWC Health Research approval (RP063/2013)

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PART B. LITERATURE REVIEW

1. Background

Paediatric HIV infection remains an important health challenge globally (1, 2). Observational studies and randomised controlled trials confirm that early initiation of antiretroviral therapy (ART) in HIV-infected infants substantially reduces mortality and acute and chronic morbidity (3-5). Furthermore, clinical evidence suggests that initiation of ART within hours to days of birth can preserve immune function and limit viral dissemination with positive consequences for growth and development (6, 7). Timeous treatment requires early diagnosis. Technological developments in nucleic acid testing (DNA/RNA PCR or a combination of the two, henceforth denoted HIV-PCR) have increased the sensitivity and specificity of *birth* HIV diagnostic assays (8, 9). Together with programmatic advances this has allowed the use of technology at scale to make such testing feasible even in some resource-limited settings.

With the evolution in the scope and sophistication of vertical transmission prevention of HIV (VTP) strategies, programmatic and therapeutic, and the overwhelming evidence of the benefits of early ART in infants, the appropriateness of the World Health Organization (WHO) recommendation of an initial diagnostic HIV-PCR at six weeks old has been questioned in some settings (9). The 2016 *WHO Consolidated Guidelines on the use of Antiretroviral Drugs for Treating and Preventing HIV Infection* included a conditional recommendation for HIV-PCR testing at birth *in addition to* existing testing algorithms (1). The *South African National Consolidated Guidelines for the Prevention of Mother-to-Child Transmission of HIV (PMTCT) and the Management of HIV in Children, Adolescents and Adults* introduced routine diagnostic testing for all HIV-exposed infants at birth in 2015 with repeat testing at 10 weeks old (10). The rationale behind this recommendation is that testing at birth would identify all neonates infected *in utero* who could then be fast-tracked to treatment. Repeat testing at 10 weeks would detect peri-natal and early post-partum infections. Ten weeks coincides with the second routine visit of the WHO's Expanded Programme on Immunization schedule (11).

2. Objectives

Programmatic data on the impact of birth HIV-PCR testing of HIV-exposed infants in sub-Saharan Africa is lacking (12, 13). This review aims to appraise published assessments of birth HIV-PCR testing programmes in low and middle income countries (LMIC), to summarise existing knowledge and identify research gaps.

There is considerable debate as to the appropriateness and cost effectiveness of birth HIV-PCR testing versus testing at other time-points in resource constrained settings, owing to the high cost nucleic acid tests. Since birth testing identifies *in utero* HIV infections only, subsequent early tests would be necessary to detect peri-natal and early post-partum infections (1, 14-16). While acknowledging these controversies, this review is confined to assessment of birth testing programmes. Some discussion of optimal time points and costing will be included in the analysis of the modelling literature.

The specific objectives of the review are to report on:

- Proportion HIV infected infants identified at birth and overall *in utero* HIV transmission rate
- Performance of the diagnostic assays
- Age of follow-up HIV-PCR
- Linkage to care/time to ART in HIV-infected infants
- Resources required for implementation of birth HIV-PCR
- Studies modelling the impact of birth HIV-PCR will be reviewed specifically

3. Concepts and Definitions

The definition of early infant diagnosis (EID) has evolved with technological and operational advances allowing us to confirm diagnosis within hours of birth, but also requiring that we extend monitoring prospectively through the entire exposure period to the end of breast-feeding (3). Mother-to-child transmission of HIV (MTCT) can occur during pregnancy, labour and delivery, and post-partum via breastmilk. Based on data from VTP randomized controlled trials, Kourtis *et al.* proposed estimates for the timing of MTCT (without any antiretroviral intervention). In a non-breastfeeding population, the majority of transmission occurs during late pregnancy and delivery (peri-natal infection;~80%) with approximately 20% occurring *in utero;* that is, during pregnancy. In populations with prolonged breast-feeding (up to 24 months), an estimated 40% of MTCT occurred during the post-partum

period, 50% peri-natal and 10% *in utero* (17). Initial drug-based VTP interventions covered the high risk peri-natal period, changing these proportions. Under the WHO option B+ PMTCT guidelines in which HIV-infected pregnant women are initiated on ART for life at diagnosis regardless of immunological or clinical stage of disease, many more pregnancies are protected by ART for longer periods. Evidence suggests that under these circumstances *in utero*, and particularly post-partum transmissions will comprise the majority of infections (9).

Definitions

In utero transmission of HIV: HIV-PCR positive in blood samples within 3 days of birth (18).

Intrapartum transmission: HIV-PCR negative in blood samples within 3 days of birth but subsequently positive (non-breast-feeding population; in the presence of breast-feeding is it difficult to distinguish from early post-partum transmission) (18).

Post-partum transmission: HIV-PCR negative in blood samples at birth and 6-10 weeks old but subsequently positive in the presence of breast-feeding (19).

Diagnostic Assays in Infants and Young Children

Maternal HIV-specific antibodies cross the placenta and enter the fetal circulation where they can persist for up to 18 months. Serological HIV assays (antibody tests) are therefore unreliable for the diagnosis of HIV infection in infants (they can be useful to determine exposure) and virological assays that detect HIV nucleic acids (Nucleic Acid Testing [NAT]) or viral antigens (e.g. p24) are required for definitive diagnosis (1, 3). HIV DNA PCR assays are routinely used in EID programmes (1). In South Africa, the National Health Laboratory Services (NHLS) uses the CAP/CTM total nucleic acid real-time PCR which detects both pro-viral DNA and RNA of HIV-1. The assay can be performed on dry blood spots (DBS) or EDTA blood samples (8).

4. Search strategy

A search of the Medline database via PubMed (National Library of Medicine, Bethesda, MD) was performed using the following strategy:

"hiv"[MeSH Terms] OR "hiv"[All Fields] OR Human Immune Deficiency Virus OR Human immunodeficiency virus

AND PMTCT OR “Mother-to-Child Transmission of HIV” OR “Prevention of Mother to Child Transmission of HIV” OR “Vertical Transmission” OR “Vertical Transmission of HIV”

AND Infant diagnosis OR “Early Infant Diagnosis” OR EID

AND “Birth Testing” OR “Birth PCR”

AND "humans"[MeSH Terms] AND English[lang] AND "infant"[MeSH Terms] NOT *toxo NOT cmv

Reports were limited to those published in English from 2008 to present, 2008 being the year in which the WHO guidelines were amended (following the findings of the CHER study (4)) to recommend treatment of all infants less than 12 months old regardless of clinical and immunological stage(20). The search was censored in November 2017.

Proof of principle and diagnostic assessments and validations of PCR assays, including point of care tests, and sampling techniques (e.g. dry blood spots) were excluded, the focus being on programmes/studies that incorporated birth HIV-PCR in the EID algorithm; not on the sampling or laboratory methods themselves. Reports from high income countries were excluded.

Study selection

Publications identified by the search were reviewed. Duplicates were removed and titles and abstracts of the remaining articles evaluated. All articles identified through this process were assessed per the objectives specified above. In addition, the bibliographies of reviews of EID identified through PubMed were reviewed to identify additional studies (3, 5, 6, 12, 13, 15, 16, 21-24).

Search results

The search yielded 13 articles published or e-published prior to November 2017 (13, 14, 25-35). Eight were excluded for the reasons outlined. Two were unrelated to HIV (28, 30); and two were evaluations of point of care assays (27, 34). One reported on the findings of a workshop held to evaluate evidence for HIV birth testing for EID (13). One paper reviewed an EID pilot programme in Tanzania which did not include birth HIV-PCR (32); nor did the VTP programme in Thailand over the period reported by Naiwatanakul *et al.*(31). Thailand introduced EID on day 1-2 of life in 2015 (13). None of the Thai references identified, including those that assessed the elimination of vertical transmission achieved by Thailand in 2015, examined EID in detail, focussing more on maternal interventions (36). They were therefore not included.

There were two studies using mathematical models to determine potential benefits and costs of HIV birth testing (14, 29). A third modelling study using Brazilian data was excluded as this assessed maternal antenatal VTP scenarios with no mention of infant testing schedules (25). Of the 13, five papers met the inclusion criteria including the modelling studies. (14, 26, 29, 33, 35). A further three were identified by searching the bibliographies of reviews (8, 19, 37). No quality criteria were applied for inclusion (Table 1.)

The two modelling reports are discussed in a separate section below (14, 29). Bibliography searches resulted in a further modelling manuscript (38). Two of the modelling studies use data from South Africa (14, 29) and the third from sub-Saharan Africa (38). These will be considered separately in section 5.8 and Table 2.

Author, year	Study period	Design	Setting	Population	Age at 1st PCR	Assay	Sample size	Transmission	Follow-up testing	Linkage to care/time to ART	Comments
Lilian, 2012 (8) Lilian, 2013 (19)	2008 - 2010	Prospective cohort study	Level 2 maternity hospital, Johannesburg. Routine VTP programme.	HIV-exposed infants meeting study criteria	Routine HIV-PCR at 6 weeks Retrospective testing of selected birth (<24h) samples	Roche Amplicor HIV-1 DNA PCR version 1.5 Roche COBAS® TaqMan® Aptima HIV-1 screening assay (Gen-Probe)	838	5.35% at six weeks; (4.08% *in utero)*	No FU of negative infants after 6 weeks	Median 16 weeks	LTFU before 6 week test; 76% of those infants who tested positive at 6 weeks could be diagnosed at birth; Could not determine final HIV status in 15%; 47% infected infants LTFU; 16% died.
Maritz, 2016 (37)	2009-2014 and 2014-2015	Retrospective aggregate data analysis comparing 2 policy periods	Reference laboratory in Western Cape Province. Routine VTP programmes.	All HIV-PCR tests within 7 days of life	0-7 days	Roche COBAS® TaqMan®	3322	3.1% overall. 4.4% under discretionary testing policy*(period 1); 2.5% under high-risk policy (period 2)	49% of negative infants regardless of testing policy (over 182 days)	Not reported	Analysis of aggregate data; Drop in yield of birth PCR in period 2 (high-risk policy); Receipt of a birth PCR reduced likelihood of returning for 6 week PCR; Some of this LTFU could be due to mortality.
Dunning, 2017 (26)	2013-2015	Retrospective cohort study	Level 2 maternity hospital, Cape Town. Routine VTP programme .	Birth testing if met high risk criteria for transmission	< 48 hours	Roche COBAS® TaqMan®	1126	4% at birth (0.5% at FU test)	73% with neg birth PCR retested; 85% with no birth test tested at 6 weeks	86% of birth diagnosed on ART by 3m. 100% six week diagnosed on ART.	Testing high-risk infants offers high diagnostic yields; Receipt of a birth PCR reduced likelihood of returning for 6 week PCR; Some of this LTFU could be due to mortality.
Technau 2017a (33), 2017b (35)	2014-2016	Prospective cohort study	Level 2 maternity hospital, Johannesburg. Routine VTP programme.	All HIV-exposed infants	Median 14 hours	Roche COBAS® TaqMan®	6377	1.4%	Active FU of positive infants at 7 days (89%); 52% negative infants returned for results at 7days	Median 8 days in 96% of positive infants	Additional resources required for counselling and follow-up; Indeterminate test results required multiple repeat tests; Only 52% negative infants returned for birth HIV-PCR results.

Table B.1. Characteristics of studies reviewed (excluding modelling studies)

ART - antiretroviral Therapy; FU - Follow-up; LTFU - Lost to follow-up; neg – negative; m - months

*discretionary testing policy refers to birth/early HIV-PCR at the discretion of the attending clinician e.g. in the presence of clinical evidence of HIV infection

Author, year	Study period	Setting	Model	Aims	Comparator groups	Simulated sample	Outcomes	Results
Lilian, 2014 (29)	2010	South Africa	Individual-based stochastic model	1. Identify the EID strategy that would maximize the number of in utero and peri-natal HIV infections diagnosed and number of life-years saved. 2. Highlight any programmatic concerns that might impact on diagnosis.	**A**. 1 x HIV-PCR: at birth, 6, 10 or 14 weeks **B**. 2 x HIV-PCR: at birth + 6, 10 or 14 weeks	240 000 HIV-exposed infants	No. of peri-natal diagnoses No. of life-years saved	Single test: 10 weeks maximized the number of HIV-infected infants identified but did not save additional life-years when compared to a single birth test. Two PCR tests: birth and 10 weeks superior to a single test at six weeks in terms of no. peri-natal diagnoses.
Chiu, 2016 (38)	2012	Sub-Saharan Africa	Excel decision-tree model	1. Evaluate the benefit of adding an HIV-PCR at birth to the existing six week time-point	**A.** 1 x HIV-PCR at 6weeks **B.** 2 x HIV-PCR at birth + 6 weeks	1 400 000 HIV-infected women	No. of infants on ART by 3 & 18m No. of deaths prevented at 12m	Birth HIV-PCR: Increase in number of infants on ART at three and 18 months and increase in survival. Greatest effect in settings with low rates of VTP uptake and facility births.
Francke 2016 (14)	2015	South Africa	CEPAC Pediatric model	1. Calculate the cost-effectiveness for each EID strategy.	**A**. 1 x HIV-PCR: at birth or 6 weeks **B.** 2 x HIV-PCR at birth + 6 weeks **C.** no EID	N/A	Incremental cost effectiveness ratios (ICER)	Single HIV-PCR strategy: six weeks was more effective clinically and financially than birth. Cost effectiveness per life-year saved: birth and six weeks testing better than six week time-point alone.

Table B.2. Characteristics of the modelling studies reviewed

CEPAC - Cost-Effectiveness of Preventing AIDS Complications; EID - Early Infant Diagnosis; no. – number; m - months

5. Results

5.1 Study designs, settings and populations

All six observational studies were based in South Africa (8, 19, 26, 33, 35, 37). In two instances, there were two papers reporting different aspects of the same study ((8, 19) and (33, 35)). Both these were prospective observational cohort studies at the same study site in Johannesburg but at different times and during different VTP guideline periods. The site, an urban secondary maternity hospital, had a strong, established VTP infrastructure with comprehensive HIV services (antenatal and peri-partum maternal testing, maternal ART, infant testing as per the relevant guidelines, and paediatric ART; dedicated staff retested women of unknown or negative HIV status postpartum before discharge, including after hours and weekend coverage (33).) I will discuss the studies as presented in both papers, not the individual publications.

The remaining two studies report retrospective cohort analyses, one based on a folder review from an urban secondary maternity hospital in Cape Town (26); and the other aggregate laboratory data from one of the two main HIV testing laboratories in the Western Cape (37). The latter included samples from across the centre's drainage area, including primary and tertiary sites, and was not limited to births occurring in a secondary hospital. The denominator did not include a sample of HIV-exposed infants in either case; HIV-PCR tests were the starting point i.e. HIV-exposed infants who did not receive an HIV-PCR were excluded.

All studies reported positive birth HIV test results and *in utero* transmission rates, and three gave data on subsequent HIV testing (8, 19, 26, 37). Three provided some measure of linkage of HIV infected infants to care (19, 26, 33, 35). The diagnostic and other tests in all the studies were performed by the NHLS which serves all the state-run health facilities in South Africa. Over the time period in which the studies were conducted (2008 – 2016) the national and regional VTP guidelines were updated four times (10, 39-42); the relevant recommendations are discussed as applicable.

5.2 Study methodology

The study reported in the papers by Lilian *et al.* (8, 19) was a prospective observational cohort study which enrolled HIV-infected and -exposed mother-infant pairs from the postnatal wards at a secondary hospital in urban Johannesburg. Dry blood spot samples were collected at birth, two, four and six week visits and stored. Initial HIV diagnosis relied on the routine HIV-PCR testing at six weeks old (as per the national guidelines (42)) which was performed predominantly at the hospital's VTP clinic or a related primary clinic managed by an HIV NGO (i.e. the six week diagnostic test was not a study procedure). At the end of the project the NHLS national database was searched for any six week HIV-PCR tests (and the results) performed on the participants to minimize the missing outcomes. HIV-infected infants entered the routine treatment service, again mainly at the paediatric HIV treatment clinic at the hospital. The VTP guidelines at the time, short-course antiretroviral treatment, comprised maternal ART if the CD4 count was less than 200 cells/ml. Women with CD4 counts greater than 200 cells/ml received daily zidovudine (AZT) from 28 weeks and single dose nevirapine (NVP) during labour. Infants received NVP at birth and four to 28 days of AZT depending on whether the mother received treatment for greater than or less than four weeks prior to delivery (42). Exclusive formula feeding was supported by the state and NGOs. Towards the end of the study (April 2010) the guidelines were updated raising the eligibility for maternal ART to a CD4 count of less than 350 cells/ml. ART or AZT were prescribed from 14 weeks gestation and infant prophylaxis changed to six weeks of daily NVP. Exclusive breast feeding was now encouraged and subsidized formula phased out.

In the infants identified as HIV-infected by the routine programme, the stored DBS samples (with two matched controls) were retrospectively tested using three different nucleic acid assays. This allowed for assessment of the technology (8) and timing of infection (19). This study was the first to assess the addition of birth HIV-PCR testing to the routine six week algorithm in use in many LMIC and has (despite its limitations) provided much of the evidence supporting introduction of birth testing to WHO and South African guidelines (1, 10).

Technau *et al.* in a prospective cohort study conducted at the same site between June 2014 and December 2016, assessed a pilot birth HIV-PCR testing programme (33, 35). (Birth HIV-PCR was introduced in the South African national guidelines in 2015 (10).) Study counsellors enrolled HIV-infected women from the postnatal wards and nurses performed the blood sampling. Results were collected by caregivers one week later. The study staff made considerable effort to contact the mothers of infants with non-negative (i.e. positive and indeterminate) HIV-PCR results and perform follow-up testing as required. Carers of infants with negative birth HIV-PCR results were not pursued if they did not collect results. ART for infected infants was initiated at the same facility. The South African VTP guidelines during this period (10) recommended WHO Option B+ for all HIV-infected women who initiated a single daily fixed dose combination tablet comprising tenofovir (TDF), emtricitabine (FTC) and efavirenz (EFV). Infants received daily NVP for six weeks and exclusive breast feeding was encouraged. By this time subsidized formula was no longer provided at government facilities. Initial diagnostic HIV-PCR remained at six weeks until June 2015 (high-risk infants were eligible for birth testing) when birth HIV-PCR for all HIV-exposed infants became national policy, with a follow-up test at 10 weeks old (10).

Dunning *et al.* described a retrospective cohort study of neonates at high risk of HIV transmission who received a birth HIV-PCR at a maternity hospital in Cape Town. Those who tested negative at birth were matched on date of birth and mode of delivery with HIV-exposed controls who were not tested at birth. The NHLS database was searched to identify subsequent test results in both groups to compare differences in presentation for follow-up testing (at six weeks as per the guidelines (26)) in infants who did and did not receive a birth HIV-PCR. The VTP guidelines at the time were the same as those at the start of Technau *et al.*, that is, WHO option B+ with birth HIV-PCR indicated only for infants who met high-risk criteria for transmission of HIV (40).

Maritz *et al.* used aggregate HIV-PCR testing data from the Western Cape NHLS database to compare presentation for follow-up testing after birth HIV-PCR during two birth testing policy periods (37). During the earlier period (January 2009 – March 2014) clinicians could request a birth test in symptomatic infants. The 2014 *Western Cape PMTCT Clinical Guidelines Update* introduced selective birth testing for those infants meeting high risk criteria for transmission (40). This second period (April 2014 – June 2015) overlapped with

that reported in Dunning *et al.* (26). Specific linking software was used to maximize probabilistic linkage between birth and subsequent HIV-PCR tests.

5.3 Participants and sample size

The sampling populations of both Johannesburg studies included *all HIV-exposed infants* and were not restricted by risk or other categories. Mothers were, however, required to provide informed consent (8, 19, 33, 35). The inclusion and exclusion criteria of the earlier study (Lilian *et al.* 2012 and 2013) further limited enrolment to women who knew their HIV status antenatally (i.e. who were not diagnosed during labour or immediately postpartum) and who planned to return to the facility for follow-up. Recruitment occurred only during working hours. Eight hundred and thirty-eight neonates were enrolled for whom an HIV status at six weeks could be determined in 710 (84.7%)(19).

Technau *et al.* enrolled mother-infant pairs outside working hours although coverage was incomplete. Of 7085 HIV-infected women, 727 (10.3%) were not screened, were ineligible (<18 years old) or did not consent. The median age of testing of 6377 infants was 14 hours (33). Dunning *et al.* identified 575 high-risk neonates who received a birth HIV-PCR. The 551 with a negative result were matched on date of birth and delivery method with 551 HIV-exposed infants who did not receive a birth test (despite some presenting with high risk criteria)(26). Maritz *et al.* assessed 3 322 infants with a birth HIV-PCR test, defined as occurring within seven days of birth (37).

5.4 Positive birth HIV-PCR results and in utero transmission

The proportion of positive birth HIV-PCR results is dependent on the timing and effectiveness of VTP ART coverage and the prevalence of risk factors for transmission in each study population. The study populations that Dunning *et al.* and Maritz *et al.* included and tested were high-risk by definition, either meeting specific criteria or being symptomatic for HIV infection (period 1 in Maritz *et al.*). In addition, Dunning *et al.* sampled a hospital population indicating a level of clinical risk above that of primary care. It is unsurprising that the percentages of positive birth tests in these studies are high, 3.8% and 3.1% respectively. These are higher than the overall transmission rates reported for the Western Cape during the period (1.9% before two months old (43)). Of note in Maritz *et al.*

is that the proportion of positive results dropped from 6% in 2009 (symptomatic infants tested at the clinician's discretion) to 1.6% in 2015 as the population eligible for birth testing expanded (routine birth HIV-PCR for all HIV-exposed infants) with a corresponding dilution of diagnostic yield/impact (37). In contrast, Technau *et al*., in a large sample of all HIV-exposed infants, reported an *in utero* transmission rate of 1.6% reflecting the reduced prevalence in a general population of HIV-exposed infants at all levels of risk(33).

In the study reported by Lilian *et al., in utero* transmission was 4.1% (8). This was determined by retrospective testing of participant stored birth DBS samples *after* a positive HIV-PCR result at six weeks or older. Transmission is high given broad inclusion criteria (and that the high-risk group of newly diagnosed women was excluded) and is probably a reflection of shorter and less comprehensive VTP ART strategies at the time (WHO Option A (42)). It has been repeatedly demonstrated, most recently in a randomized controlled trial, that ART is more effective in preventing vertical transmission of HIV than single or dual antiretroviral therapy (44). South African VTP guidelines from 2013 were either Option B or B+ thus reducing transmission risk over the periods in which the other studies were conducted.

5.5 Performance of the assays

As maternal ART and infant post-exposure prophylaxis have become more effective, and with the emphasis on very early testing and treatment of infected neonates, concerns have been raised regarding the performance of diagnostic nucleic acid assays (9, 39, 40, 45). A recent systematic review calculated a pooled sensitivity of 69.3% for birth HIV-PCR in the presence of maternal and infant ART although the quality of the evidence was low; specificity was more reassuring at 99.9% (quality of evidence intermediate-high) (24). These figures included data from the study reported by Lilian *et al.* (2008-2010; sensitivity 76.3%), (8) and data from an earlier French cohort analysis (40) conducted between 1994-2006 (sensitivity 56.7%). Given the advances in nucleic acid testing technology, the time periods are relevant. The authors of the review argued that the low sensitivity reflects the inability of birth testing to identify peri-natal infections as opposed to the poor performance of the assays themselves (24) which emphasizes the need for additional testing during infancy.

One of the concerns highlighted by Technau *et al.* was the high numbers of errors (0.9%) and indeterminate results (0.3%) from birth EID and the additional follow-up and repeat testing these necessitated. An indeterminate result is one that is valid but inconclusive – the assay detects the target (e.g. HIV nucleic acid sequence) but the signal is below the threshold required to confirm a positive result (46). The proportion of these non-negative results could increase with the introduction of point of care (POC) tests which are currently less sensitive and more prone to error that conventional laboratory assays (27). It is hypothesized that infant post-exposure prophylaxis and maternal ART in breast milk could reduce HIV viral load below the detectable levels of routine nucleic acid tests, giving an indeterminate or low positive result. While Mallampati *et al*. found no evidence for this in their review, albeit in the face of limited evidence (24), observational studies suggest otherwise. Clinicians face the growing challenge of interpreting indeterminate or low-positive test results (8, 47-49).

Even in 2008-2010, the single dose NVP and daily AZT infant prophylaxis seemed to affect the performance of three diagnostic assays (Lilian *et al*.) Some samples (n=4) from the two week time point tested negative in individuals infected *in utero* (i.e. with positive birth and six week HIV-PCRs). The authors hypothesized that the infant NVP may be the cause; reducing the viral load below the threshold of detectability in all three assays reviewed (8). A more recent pooled analysis in non-breastfed populations demonstrated that the type and duration of infant prophylaxis did affect time to positivity of HIV-PCR. The probability of a positive birth test was lower if women had received no prophylaxis (but similar at 14 days regardless of maternal intervention). This reflects the effect of VTP regimens on intrapartum transmissions. i.e. there is a greater proportion of *in utero* infections (diagnosable at birth) in treated women (45).

In the study reported by Technau *et al.*, considerable resources were dedicated to tracing infants with non-negative results and repeated assays were often required to confirm the diagnosis. Of 19 infants with indeterminate results, seven (36.8%) ultimately received a positive diagnosis, delayed by a median of 30 days; and four (21.0%) infants died before a definitive status could be established. Those neonates with a definitive positive HIV-PCR result at birth had significantly higher viral loads than those whose initial test was indeterminate (35). A retrospective laboratory data analysis of indeterminate and follow-up

HIV-PCR results in infants in Cape Town (median age 45 days), also under Option B+ VTP regimens, reported indeterminate rates of 3.2%; 64.1% of infants with initial indeterminate outcome subsequently tested positive a median of 28 days later. The study did not report on loss to follow-up after the initial indeterminate result (48). Dunning *et al.* and Maritz *et al.* report the proportion of indeterminate/equivocal birth HIV-PCR results as 0.3% and 1.2% respectively (26, 37). Neither discusses these in detail or reports on follow-up results.

5.6 Return for follow-up testing

By definition birth HIV testing will only identify *in utero* infections. While *in utero* and post-partum transmission may account for a larger proportion of infant HIV infections than peri-natal infections under more effective VTP regimens (29, 45), repeat testing during early infancy is essential to detect intra-partum and early post-partum infections. The most recent iteration of the South African and Western Cape VTP Guidelines (2015; (41)) specify repeat HIV-PCR at 10 weeks old following a birth test. In most of the studies reviewed here, a birth HIV-PCR was added to the existing recommended test at six weeks old.

Loss to follow-up (LTFU) between birth and six weeks has been repeatedly high-lighted as compromising the VTP cascade in many settings in the absence of birth testing (3, 16, 23); indeed, it is one of the arguments in support of birth testing (9). There is, however, a concern that receipt of a negative birth test result may falsely reassure the caregiver resulting in reduced presentation for follow-up testing. The risk may be even higher with the delay of repeat testing beyond the previously accepted six week time-point. Unfortunately, South African data suggest that receipt of a birth HIV-PCR does reduce likelihood of receiving a follow-up test (26, 37, 50) and there is concern that the policy may undermine the existing part-partum EID service which reached over 80% of exposed infants in 2014 (12). One EID mathematical model suggests that the cost benefit (in terms of USD per life-year saved) of adding a birth HIV-PCR to the six week test is lost if more than 37% of exposed-infants fail to return for repeat testing (14).

Lilian *et al*. report LTFU at six weeks of 17.5% (with telephonic tracing). These participants did not receive a birth test result as the stored DBS were tested retrospectively. Ninety-three percent of those who did present for testing returned for results (26 [4.3%] were HIV infected.) HIV status was established on 19 of those LTFU by testing stored study DBS

samples (from birth, two and four weeks). Overall, only 67% of the total number of infants tested received results at a median age of 10.6 weeks (19). It is important to note that this comprised a study group, who had consented to participate and who were contacted by phone in the event of missed visits.

The sample reported by Technau *et al.* also comprised a study population, and only 52% of those with negative birth HIV-PCR tests returned to collect the results one week later (35). Considerable resources were committed to engaging in care those with non-negative results (i.e. positive and indeterminate) and the negative remainder were not followed up. The rates of routine HIV-PCR at six or ten weeks were not examined. Those with indeterminate results required considerable input.

In the analysis by Dunning *et al.* 73% of infants with a birth HIV-PCR presented for routine testing at six weeks versus 85% of those with no birth PCR (adjusted OR 0.60 [95% confidence interval 0.41 – 0.86]); the risk persisted across demographic and clinical subgroups (26). The latter figure (i.e. 85%) is closer to that in the study described by Lillian *et al.* It is relevant to note that in this earlier study (Lilian *et al.*) women did not receive a birth result as samples were tested retrospectively (19) whereas the caregivers hospitalized infants reported in Dunning *et al.* may well have received a negative HIV-PCR result before discharge. Maritz et al. report that only 49% of those tested at birth ever received a subsequent test, regardless of testing policy; this dropped to 43% if they restricted follow-up tests within the guidelines window of 6-10 weeks. No non-birth test comparator is presented (37).

Lilian *et al.* report a transmission rate of 5.4% at six weeks. *In utero* transmission was 4.1%. When one excludes those testing positive at birth, transmission rate at six weeks was 1.3% (8). The drop in *in utero* transmission four years later (to 1.6%) observed at the same site by Technau *et al.* probably reflects advances in the antenatal VTP programme (33).

In the cohort presented by Dunning *et al.* testing at the six week time point yielded a far lower proportion of positive results regardless of whether a birth HIV-PCR had been performed or not, 0.5% and 0.4% respectively (26), demonstrating the high yield with targeted birth testing. Those infants who tested positive at birth were excluded from the later test and those not tested at birth were more likely to be at lower risk of transmission.

The latter two studies are limited in that they are unable to ascertain reasons for LTFU, particularly mortality. Under the discretionary and high-risk testing policies, infants receiving HIV-PCR at birth were more likely to be born in hospital or referred immediately after birth and by definition met high-risk criteria including clinical symptoms, low birth weight and prematurity all of which increase neonatal mortality. It is likely that a proportion of those LTFU may have died (from any cause) before repeat testing. (Both Johannesburg studies report deaths prior to diagnosis and ART (19, 35)).

5.7 Linkage to HIV care

Three studies discuss linkage of HIV-infected infants to care (19, 26, 35). One of the key findings reported by Lilian *et al.* which has been used to promote birth HIV testing agenda was that an initial test at six weeks old resulted not only in loss to follow-up of *in utero* infected infants but in delayed initiation of ART in those following the EID algorithm at the time (median age at ART start, 16 weeks). (The initial CHER findings, recommending early infant ART before seven weeks old (4) and the Bourne data demonstrating the peak in South African infant mortality at 2-3 months old (50) were published early in the course of the study. i.e. there was increasing awareness of the high early mortality of infant HIV infection and that early diagnosis and treatment could reduce this.) Four infants were LTFU between diagnosis and ART, and six died, three soon after initiation. Only 37% of HIV positive infants remained in care at one year, but the majority were virally suppressed. There was considerable attrition after starting ART (19).

Dunning *et al.* did not discuss time to ART but reported viral suppression (which was determined from NHLS laboratory results). Eighty-six percent of neonates diagnosed at birth had evidence of ART versus 100% if the first HIV-PCR occurred later (there were only four in this group). Again it is not clear whether the attrition was due in part to mortality in the birth group. Infants who received a birth test took longer or failed to achieve viral suppression (26). This may be due to viral dynamics and the timing of infection (i.e. *in utero* versus peri-partum); or to social factors which could influence both transmission risk (e.g. associated with limited or no antenatal care or ART) and infant ART adherence.

In Technau *et al.*, active outreach for infants with non-negative birth HIV-PCR tests ensured that 96% of infected infants were traced and initiated on ART, the majority within a median

of eight days at the hospital. Extra staff were required for outreach, counselling and administration. An additional seven infants (7.1%) were confirmed as having initiated treatment at other sites between 40-99 days (considerably older)(33).

A retrospective study in the Western Cape between 2005 and 2011 using laboratory data to link positive HIV-PCR results to subsequent viral load assays (viral load as a marker of having attended for HIV care) found that 65% of infected infants received at least one follow-up visit. By 2010 this was 71% with a median time to follow-up of 33 days. The sample here is larger comprising 6 322 infants (51).

5.8 Modelling the timing of Early Infant Diagnosis

Faced with operational challenges and attrition of exposed infants from EID programmes, as well as the clinical consequences of missed or delayed diagnosis of vertical HIV infection, and with therapeutic, technological and programmatic advances, several groups have presented mathematical models to inform optimal time-points for EID in LMIC. Since virological assays are expensive and birth PCR necessitates additional testing time-points, much of the focus has been based on cost relative to diagnoses made and life-years saved. I identified three papers that modelled alternative EID testing strategies and will discuss these here (Table 2). Two models were based on South African data (14, 29) and the other used data from sub-Saharan Africa then applied the model to Kenya and Swaziland specifically (38). All used laboratory-based nucleic acid assays for diagnosis, with the attendant delays. The introduction Point of Care (POC) testing may impact the models' projections: since results are available within hours the prolonged turn-around times (TAT) sending samples from testing site to laboratory and results back to the site would become irrelevant. However, assumptions regarding the sensitivity and specificity of laboratory-based nucleic acid assays cannot be applied to POC tests.

The studies differed in terms of specific research questions and objectives, and in terms of the design and level of complexity of the mathematical model applied.

Lilian *et al.* is the earliest published attempt to model birth HIV-PCR testing (29). They present an individual-based stochastic model designed to simulate antiretroviral prophylaxis, infant feeding and health outcomes in 240 000 HIV-exposed infants. An

individual-based model can accommodate changes in the parameters over time and is better able to simulate long term outcomes (as opposed to a decision-tree analysis, for example [see Chiu *et al.* (38)]). The study aimed to identify the EID strategy that would maximize the number of *in utero* and peri-natal HIV infections diagnosed and number of life-years saved; and to highlight any programmatic concerns that might impact on diagnosis. The standard assumptions were based on the 2010 South African VTP guidelines (WHO option A; (41)) although option B was modelled as one scenario. Rates of HIV-PCR uptake were based on South African sources and the sensitivity of HIV-PCR at six weeks old was assumed to be reduced in the context of prolonged daily NVP prophylaxis. A single HIV-PCR at different time points (birth, six, 10 or 14 weeks) and two HIV-PCRs (birth *and* six, 10 or 14 weeks) were compared. A single birth HIV-PCR offered no advantage over single test at six weeks old in terms of number of diagnoses made. A single test at 10 weeks old maximized the number of HIV-infected infants identified but did not save additional life-years when compared to a single birth test (owing to death before diagnosis at 10 weeks). When two PCR tests were considered, testing at birth and 10 weeks old were superior to a single test at six weeks (under the guidelines at the time) with a 30% increase in HIV diagnoses and 61% increase in life-years saved. This advantage remained under different feeding and VTP programme scenarios. Cost-effectiveness analysis was not performed (29).

Francke *et al*. also used an individual-based model and South African data (14). Their standard parameters were based on the 2015 South African VTP guidelines (WHO Option B+; (10)) and assumed expanded ART coverage in pregnant women and 80% breastfeeding prevalence. The Cost-Effectiveness of Preventing AIDS Complications (CEPAC) Pediatric model was used to compare four EID testing strategies: no EID; a single HIV-PCR at birth or six weeks; and two HIV-PCRs at birth and six weeks. The CEPAC-Pediatric model is an adaptation of the microsimulation model of pediatric HIV disease expanded to account for transmission and diagnosis. It is a complex model able to accommodate infants from birth to death. Survival, HIV-related healthcare costs and life expectancy were used to calculate the incremental cost-effectiveness ratio (ICER) for each EID strategy. When a single HIV-PCR strategy was assessed, the six week time-point was more effective clinically and in terms of cost than birth. Cost effectiveness per life-year save favoured birth and six weeks testing above six week time-point alone. But, survival benefits were lost if 37% of infants did not

return for follow-up testing; and increasing the proportion of results returned and HIV-infected infants linked to care with the single test at six weeks had a greater impact on survival than the addition of a birth test. The authors recommended strengthening the six week testing programmes before the addition of the birth HIV-PCR (14).

Chiu *et al.* used the least complex model (Microsoft Excel decision-tree model) to evaluate the benefit of adding an HIV-PCR at birth to the existing six week time-point for 1 400 000 HIV-infected pregnant women and their infants in sub-Saharan Africa (38). This differs from the South African studies in scale and range: antenatal HIV prevalence, maternity and infant care infrastructure, proportion of facility-based births, and HIV and VTP treatment programmes vary widely across the region. Sensitivity analyses were therefore performed using low and high estimates of facility-based births, VTP strategies and uptake, and infant testing. The model was also applied under two country-specific conditions, Kenya and Swaziland. The outcome (benefit of addition of a birth test) was assessed as the number of HIV-infected infants initiated on ART by three and 18 months and the number of HIV-related deaths prevented at 12 months. Addition of a birth HIV-PCR increased the number of infants on ART at three and 18 months with a corresponding increase in survival. Not surprisingly, the rates of facility births and enrolment of infected infants into HIV care had the biggest impact on the birth testing strategy. Even with a facility birth rate of 43%, the number of infants on ART at three months increased by 543% with birth HIV-PCR. Uptake of VTP services was also important – the poorer the uptake, the greater the rates of transmission and the greater the impact of birth testing in diagnosis and referral for ART. Thus addition of a birth HIV-PCR would have the greatest effect in settings with low rates of VTP uptake and high rates of facility births (the country example of Kenya in this study.)

All mathematical modelling studies are limited by the chosen model's assumptions. These are based on the available data which in the field of VTP may soon become outdated. Lilian *et al*. based their analysis on the South African 2010 VTP guidelines; Francke *et al.* on the 2015 iteration. Assumptions of HIV-PCR assay sensitivity at different time-points may be inaccurate in the face of prolonged *in utero* and postnatal exposure to ART. Point-of-care HIV-PCR assays will reduce (eliminate) turnaround time for results but may compromise sensitivity. The predictions of the models are context specific. Cost effectiveness of two HIV-PCRs, birth and six or 10 weeks, is dependent in the risk of vertical transmission, rates of

linkage of infected infants to care and attrition of infants who tested negative at birth and who fail to return for follow-up testing. Given the high rates of facility-based births in South Africa the data may not be generalizable in other contexts; a point highlighted by the findings of Chiu *et al* (38).

5.9 VTP implementation gaps highlighted by the literature

While documenting significant improvements in VTP programmes, reduced transmission of HIV and improved access to care, the papers reviewed highlight persistent gaps as well as newer challenges. The majority of infants tested were born at hospital. Both Rahima Moosa Mother and Child Hospital in Johannesburg (8, 19, 33, 35) and Mowbray Maternity Hospital in Cape Town (26) have well-established and maintained VTP programmes. Even when looking at the aggregate data (37), despite the greater than 20-fold increase in the number of birth HIV tests performed when high risk testing introduced in 2014 in the Western Cape, the majority of these tests were done at obstetric hospitals (74%). How such systems operate at primary care facilities and in rural areas is unknown but they are unlikely to be as well-resourced or as efficient.

Despite the robust VTP infrastructure in Johannesburg and the resources attendant to the cohort studies, attrition remained significant in the absence of 'active outreach' (telephone calls and home visits; K Technau, personal communication). Highlighted in all studies, is the fragmentation of health care in South Africa and the uneven distribution of skills and resources. Routine infant immunization and care (and VTP follow-up) occur neither at hospitals nor at the midwife obstetric units (MOU) which offer primary care delivery services. Carers are directed to local clinics which are often managed by a different branch of government; there are limited electronic medical records systems at the sites and patient identifiers can be multiple and fluid. Identification of HIV-exposed infants is usually dependent on caregiver report or completion of the Road to Health Book, a client-retained record of growth, immunizations and clinic visits. Concerns of inadvertent disclosure and stigma undermine accurate completion of these books (43). Given that birth HIV-PCR detects only *in utero* infections by definition, repeat testing at 10 weeks is essential. The projected savings (in terms of cost and life-years) achieved by the introduction of birth testing will be undermined by failure to present for follow-up (14).

Both Western Cape studies suggest that targeted birth HIV-PCR may undermine the follow-up repeat testing (26, 37). Unfortunately Technau *et al.* did not examine this. It is possible that the contributors to the high risk indications for birth HIV-PCR in these studies are the same factors that influence return to care post-partum. That is, that the same women who demonstrated poor health seeking behaviours antenatally (present late or not at all for antenatal care; were tested late in pregnancy and received ART for limited periods; failed to suppress on treatment; or may have had lifestyles that put them at risk for premature labour and low birth weight infants – smoking, alcohol use, other illnesses) found difficulty in managing the follow-up requirements for their infants. The introduction of *universal birth testing of HIV-exposed neonates* may re-adjust the numbers presenting for repeat testing after receipt of a negative birth HIV-PCR. It is likely however, that a group of women will remain at high risk of vertical transmission despite improvements in the VTP programme and the factors, many of them social and economic, that place them at risk of transmission are the same ones that compromise the health care of their babies.

There is continued debate at to the optimal timing of infant HIV testing particularly in resource limited settings where the cost of the virological assays can be prohibitive. The modelling studies have attempted to address this. The South African Department of Health has committed to funding two early HIV-PCRs (10). With the high rate of facility-based deliveries, models suggested tests at birth and 10 weeks would be optimal. However, Technau *et al.* demonstrated considerable human resources devoted to follow-up not only of infants with positive birth HIV-PCR results but also those with indeterminate results. These infants required numerous repeat tests contributing to additional costs (35). These data appeared too late to be included in the modelling analyses. An early (2004 – 2010) South African study of 718 children with unconfirmed positive HIV-PCR tests found 40 to be negative giving a positive predictive value for the HIV-PCR in children under 18 months of 94.4%. The authors demonstrated loss of positive predictive value as MTCT rates decreased i.e. prevalence of vertically infected infants decreased (44). More recently, Maritz *et al.* using a dataset of 38 043 first HIV-PCR results demonstrated that the proportion of non-negative results reported as indeterminate *decreased* as VTP regimes intensified (11.5% under the current Option B+ guidelines) and that more indeterminate infants tested positive on repeat testing (48). This was also the case in Technau *et al* (35).

Apart from Maritz *et al.* who used aggregate laboratory data, all the studies presented were based at urban maternity hospitals with established VTP programmes. The two prospective studies were limited to the *same* site in Johannesburg. Generalizing these findings to South Africa more broadly may be inappropriate. They offer no insight into how the system would operate in the primary care setting or in rural areas.

5.10 Methodological challenges in the literature

Both prospective studies were based at the same centre and the samples could be biased towards women attending such a large urban facility (8, 19, 33, 35). Birth testing coverage was high due to project resources. Caution should be exercised in generalizing the results. In addition, both were subject to losses to follow-up and it was not possible to determine the proportion of LTFU due to death. The study report by Lilian *et al.* was biased towards identification of *in utero* diagnosis since all birth specimens were available but later sampling was incomplete (19).

Both Dunning *et al.* and Maritz *et al.* relied on laboratory data accessed through the NHLS and/or archived in the NHLS Corporate Data Warehouse. The NHLS performs all pathology tests in the public sector in South Africa representing approximately 80% of the population (12). Data from these sources are dependent of the quality of routine entry. Nationally, the NHLS data lack unique identifiers making de-duplication difficult. i.e. it is not possible to accurately determine the *number of individual infants* tested, only the number of tests. Some individuals may be counted more than once and true vertical transmission risk cannot be determined; the total number of HIV-exposed infants is unknown and cannot be used as the denominator. (12). This problem is less apparent in the Western Cape (Dunning *et al*. and Maritz *et al.*) where there has been an attempt to implement the use of unique identifiers throughout the Provincial health services. However, the system is not faultless; in addition, there is often a delay in issuing the infant unique numbers and HIV-PCR may be recorded under the maternal identifier. Although Maritz *et al.* made use of sophisticated linking software (37) they did present aggregate data.

The limitations of the mathematic models are discussed above (Section 5.8).

6. Limitations of the review

Given the recent introduction of birth HIV-PCR programmes in LMIC there are limited data available on the impact of such strategies in routine settings. Assessments are limited to South Africa where birth HIV testing of high-risk infants has been recommended since 2013 (with admittedly limited implementation) and routine testing of all exposed neonates included in the 2015 VTP guidelines (10). No studies have assessed the impact of birth testing strategies, (in terms of guideline adherence and operational impact) in primary health settings, all have been urban hospital-based where women are unlikely to be discharged within hours of delivery as occurs at primary facilities. South Africa has high rates of institutional deliveries (52) and an efficient laboratory network in the urban areas. Operational data on the implementation in rural areas is lacking.

The search was restricted to articles in English catalogued on PubMed. This excluded potential studies in LMIC where English is not the historical medium of research (Brazil, Russia). It was difficult even to determine the EID testing regimen in Brazil (not attempted for Russia).

7. Areas for further research

This review identified the following under-researched areas:

1. The extent to which guideline recommendations are implemented in different settings (e.g. urban/rural; different levels of care.)

2. Assessment of the (unanticipated) resource and cost implications of follow-up and repeat testing in those infants with indeterminate non-negative birth HIV-PCR results.

3. Whether follow-up repeat HIV-PCR testing at 10 weeks old improves as the new guidelines become routine at facilities and why infants to do not receive this test. What strategies could be implemented to improve fidelity with the VTP testing guidelines?

4. On-going appraisal of linkage to care (and ART initiation) of HIV-infected infants.

5. With a view to the elimination of MTCT in South Africa, assessment of associations and risks that predict ongoing vertical transmission in the context of option B+, and possible interventions.

8. Conclusions

It is possible to implement routine birth HIV-PCR testing of all HIV-exposed neonates in a large urban secondary hospital in South Africa. Within this context, ART initiation is indeed expedited. However, effective implementation required increased human resources and enhanced record keeping. Indeterminate results are not uncommon and require additional resources for follow-up and repeat testing. This should be considered in future cost analyses. In the context of the current South African VTP programme infants indeterminate results require follow-up and the majority will be confirmed HIV-infected.

The literature highlights the difficulties arising from the fragmentation and historic underfunding of the South African health care system: incomplete records, lack of electronic linkage, failure to communicate and follow policy.

9. Postscript: publications subsequent to November 2017

Prior to submission of this dissertation but after November 2017 an additional relevant article was published ahead of print (53). Moyo *et al.* draw on NHLS aggregate HIV-PCR testing data for the whole of South Africa to assess the first year of implementation of the birth HIV-PCR for all HIV-exposed infants policy. They report that monthly birth tests increased from 39% to 93% between June 2015 and May 2016, with a rapid drop in the number of six week tests (from 82% to 19%) and only a modest rise in 10 week testing (from 13% to 48%). This would seem to confirm the Western Cape findings discussed above that the introduction of an HIV-PCR test at birth has undermined the required follow-up testing (26, 37). The authors believe that the poor uptake of the 10 week test is due to poor implementation of the new VTP guidelines since attendance of the 10 week immunization visit is high (53). This loss to the VTP continuum (52%) is well above the modelling threshold for loss to follow-up testing (37%) over which addition of a birth HIV-PCR ceases to be cost effective (14).

With the increase in sample size the number of positive HIV-PCR tests declined with an *in utero* transmission rate of 1.1%. The high rates of HIV infection in pregnant women mean that the absolute number of HIV-infected infants remains high (247/100 000 live births) despite the low transmission rate (53).

The limitations of the NHLS database are described above (section 5.10). In addition the authors had to estimate the numbers of HIV-exposed infants (the denominator), based on maternal antenatal HIV infection rates in order to assess coverage of birth tests. While results are presented by province there are no data on the urban/rural or primary versus secondary/tertiary care distribution of testing. The gaps identified above remain.

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PART C: MANUSCRIPT

1. Title page

Neonatal and infant diagnostic HIV-PCR uptake and associations during three sequential policy periods in Cape Town, South Africa: a longitudinal analysis.

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2. Abstract

Introduction: To strengthen the early infant diagnosis (EID) programmes and timeously identify and treat HIV-infected infants, birth HIV-PCR has been recommended in the Western Cape, South Africa since 2014. Operational data on the implementation of such programmes in low- and middle-income countries are limited.

Methods: Utilizing the electronic records platform at primary care facilities, we developed an electronic register which consolidated obstetric and HIV-related data, allowing us to track a cohort of HIV infected/exposed mother/infant dyads longitudinally from antenatal care through delivery to infant HIV-PCR. We assessed guideline implementation and impact on EID during three periods reflecting sequential EID policies in a referral chain of facilities in Cape Town (primary to tertiary care). Birth HIV-PCR was indicated in period 1 if symptomatic; period 2 if meeting high-risk criteria for transmission; and period 3 for all HIV-exposed neonates.

Results: We enrolled 2012 HIV-exposed infants; the majority (89.2%) had at least one HIV-PCR. The proportion tested at 6-10 weeks old dropped from 92.9% during period 1 to 80.2% in period 3. The proportion of infants receiving birth HIV-PCR increased, peaking at 60.5% during period 3. The majority of birth tests were performed in hospital versus primary care regardless of policy period. Almost half of all infants (47.9%) had at least one high-risk criterion for vertical infection; of these, 39.7% had a birth test. Infants with more risk factors were more likely to have birth EID. Receipt of a birth HIV-PCR significantly reduced the likelihood of receiving a follow-up test at 6-10 weeks, even after adjusting for potential confounders (aOR 0.18 [0.12 – 0.26]). The proportion of positive birth tests was highest (2.9%) when birth tests were restricted to infants meeting high-risk criteria, with a low proportion positive for the first time at 6-10 weeks. During period 3, the proportion positive at 6-10 weeks was high (2.4%), highlighting the importance of follow-up to detect intra-partum and early post-partum infections.

Conclusions: Over all policy periods, EID guidelines were incompletely implemented across all levels of care but especially in primary care. Birth HIV PCR reduces return for follow-up testing which is critical for the effectiveness of the programme.

3. Introduction

The introduction of birth HIV-PCR for HIV-exposed infants as part of early infant diagnosis (EID) has been promoted as a means of maximizing the effectiveness of EID programmes by increasing testing coverage, and identifying *in utero* HIV-infected neonates allowing early initiation of antiretroviral therapy (ART)(1). The World Health Organization (WHO) included a conditional recommendation in the 2015 vertical transmission of HIV prevention (VTP) guidelines for a diagnostic nucleic acid test around birth (0-2 days), in addition to routine HIV testing at 4-6 weeks, either for all HIV-exposed infants or for those determined to be at high risk of vertical infection (targeted birth testing)(2).

It has been suggested that birth HIV-PCR will address some of the shortcomings of the 4-6 week testing time-point which is standard in many VTP programmes in low- and middle-income countries (LMIC). In 2015 operational delays with attrition at all points along the EID continuum, resulted in only an estimated 51% of HIV-exposed infants receiving an HIV-PCR before two months old in the WHO 21 priority countries (3). Even fewer initiated ART (4). Modelling studies suggest that the addition of birth HIV-PCR to testing algorithms would increase the number of infants diagnosed with HIV and therefore life-years saved (5, 6).

Since birth HIV-PCR cannot detect infections due to intra-partum and post-natal transmission, a subsequent early HIV test would be required (e.g. at 6-10 weeks). Indeed, models suggest that the clinical benefits of adding birth HIV-PCR may be eliminated if loss to follow-up (LTFU) at subsequent testing times is ≥37% (5). Given the cost implications of additional nucleic acid assays and the often poor implementation of current EID guidelines, there is debate as to the optimal number, and timing, of infant diagnostic tests (7, 8). This has prompted calls for operational data to evaluate birth HIV testing in routine settings in LMIC (7).

In 2015, the South African (SA) National Department of Health introduced birth HIV-PCR for all HIV-exposed infants, with a second test at 10 weeks old, becoming the first national programme to do so in sub-Saharan Africa (9). In the Western Cape a policy of targeted birth testing had been implemented since August 2014.

We aimed to examine the uptake of infant HIV testing under three different EID policies in a referral chain of facilities in Cape Town (from primary care to district, secondary, and tertiary level hospitals) using prospective, longitudinally-collected individual patient data. We assessed adherence to provincial EID guidelines, the yield of HIV-PCR at birth at the sites, and the impact on presentation for follow-up testing.

4. Methods

This study formed part of an implementation science project aimed at assessing VTP coverage and effectiveness with an active surveillance system in the form of an electronic register (e-register). Using the digitized medical record platform in primary care facilities, the e-register prospectively consolidated routinely collected clinical data from paper-based obstetric and HIV registers. HIV-associated laboratory data and ART prescriptions were integrated.

Setting and Participants

This was a prospective cohort study of HIV-exposed live infants of women who attended antenatal care and/or delivered at Mitchell's Plain Midwife Obstetric Unit (MPMOU), an urban primary care facility, and its referral centres in Cape Town, SA. Uncomplicated vaginal deliveries were managed by midwives at MPMOU. Approximately half of all women were referred to hospital either during pregnancy or intra-partum. MPMOU referred to Mitchell's Plain District Hospital (with operating theatres), Mowbray Maternity Hospital (level 2 with neonatal ICU facilities), and Groote Schuur Hospital (level 3 with adult and neonatal ICU facilities) based on standardized criteria.

Between February 2014 and December 2015, all women presenting to MPMOU for antenatal care with an expected or actual delivery date before June 2016, regardless of HIV status, were enrolled. Women who presented for the first time in labour (i.e. received no antenatal care) were enrolled until June 2016. Deliveries were followed through December 2016.

WHO Option B+ was the VTP policy in place over the study period (10-12). Women of negative/unknown HIV status were offered HIV testing (rapid assay) at their first antenatal visit, during the third trimester, and during labour/immediately post-partum. HIV-infected

women initiated life-long ART. HIV-exposed infants received 6-12 weeks of daily nevirapine (NVP) depending on whether the mother had received ≥8 weeks of ART before delivery or not. In 2015, these guidelines were amended so that neonates at low risk of vertical transmission received six weeks of NVP, whereas NVP was extended to 12 weeks for high-risk infants with zidovudine added for the first six weeks (10).

EID Testing Periods

The Western Cape EID guidelines changed twice during the course of the study, giving three EID policy periods (10-12). During *period 1* (May 2013-July 2014), birth HIV-PCR was offered in addition to the routine six-week test at the discretion of the clinician where there was clinical suspicion of HIV infection (11). During *period 2* (August 2014-November 2015), additional birth HIV-PCR was indicated in the presence of defined high-risk criteria for vertical transmission (Table 1)(12). During *period 3* (from December 2015) birth HIV-PCR was indicated for *all* HIV-exposed infants regardless of transmission risk, with an additional test at 10 weeks old in place of six weeks (10).

Maternal Factors	Infant Factors
Diagnosed with HIV after 28 weeks gestation	Born before 37 completed weeks of gestation
HIV seroconversion during pregnancy	Birth weight below 2500g
Less than 12 weeks of ART before delivery	
Plasma viral load greater than 1000copies/ml	

Table C.1. High risk criteria for vertical transmission of HIV(12)

Procedures and Measurements

The e-register provided a single longitudinal record for each mother-infant dyad and included HIV testing and ART history from first antenatal visit through to infant HIV-PCR with database closure in June 2017. Results of maternal viral load (VL) performed during pregnancy or within two weeks of delivery were included. A woman was considered to seroconvert during pregnancy if she tested positive for HIV after an initial negative antenatal result. Infant HIV-PCR tests were deemed “birth tests” if they occurred within seven days of birth and as “6-10 week tests” if they occurred between four and 14 weeks old. This window included the follow-up testing time-points across all periods.

Linking maternal and infant data

According to standard procedures at MPMOU, key delivery elements were entered by midwives onto a digitized medical records system generating an infant identifier which linked the mother-infant pair. A linked infant folder number was similarly generated at the hospitals. The sample was limited to those mother-infant pairs that could be linked (96.6% live-born HIV-exposed infants in the cohort).

Analysis

Analysis was performed using STATA v.15.0 (Stata Corporation, College Station, Texas, USA). Continuous variables were summarized using means and confidence intervals (CI) or medians and interquartile ranges (IQR) for normally and non-normally distributed variables respectively. Categorical variables were described using proportions, and frequency tables used for comparison. Significance was tested using a two-sample t-test or Wilcoxon rank-sum test depending on the distribution for numerical data and the $\chi 2$ test or Fishers Exact test for categorical data. The predictors of follow-up HIV-PCR were assessed using logistic regression. Multivariate models were fitted including known or suspected risk factors for the primary outcomes.

Ethics

The study was approved by the University of Cape Town Human Research Ethics Committee and the Provincial Government of the Western Cape Department of Health Research.

A waiver of consent was granted for the e-register since the data were collected as part of routine care by the health services and entered on the provincial medical records platform falling within formal protection policies.

5. Results

We included 2012 HIV-exposed infants, 272 (13.5%) in period 1, 1391 (69.1%) in period 2, and 349 (17.4%) in period 3. The proportion of infants receiving birth HIV-PCR increased over the study period with rapid increases following guideline changes (Figure 1). Median age at birth HIV-PCR was zero days (IQR 0 – 1.4).

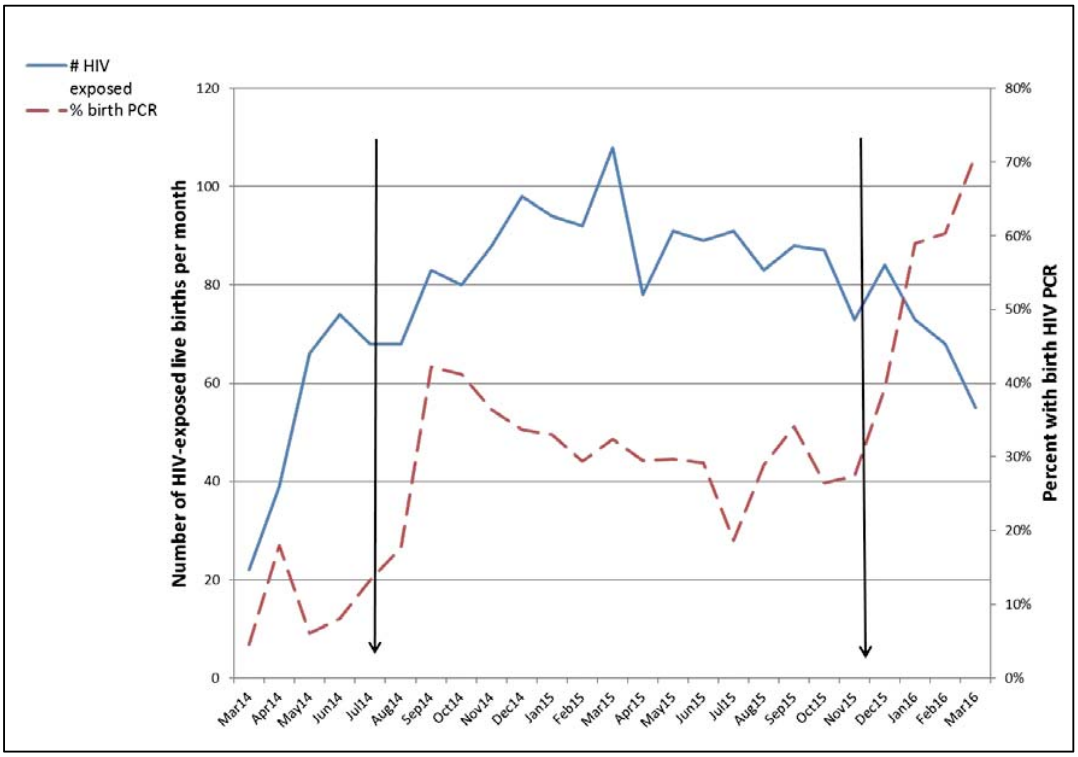

Figure C.1. The number of HIV-exposed infants and the percentage receiving birth HIV-PCR during the three policy periods. Change in EID policy is indicated by the black arrows. Generation of infant numbers at MPMOU was poor at the start of period 1, affecting linkage of infants and their mothers. Some exposed neonates were therefore not included. Few infants were born to enrolled women after March 2016 (antenatal recruitment having ceased in December 2015).

Risk factors

Over the study period, 47.9% of mother-infant pairs had at least one risk factor for vertical HIV transmission (Table 2). Viral load was >1000 copies/ml in 201 (14.9% of the 1346 (66.9%) women in whom VL was available.) The number of risk factors per infant remained stable over time but the proportion of HIV-exposed infants whose mothers were diagnosed with HIV during pregnancy (including seroconversion) decreased from 3.7% in period 1 to 1.4% in period 3. Similarly, the proportion of women who received <12 weeks of ART prior to delivery dropped: 28.3% in period 1, 21.5% in period 2 and 12.6% in period 3. Time on ART prior to delivery increased from a median of 20.1 weeks (IQR 10.6–39.9) to 21.1 weeks (12.1–85.7) and 25.3 weeks (12.6–88.9), in periods 1-3 respectively. By mid-2016, over 40% of HIV-infected women had conceived on ART. The percentage of infants born <37 weeks

gestation or with birth weight <2500g remained stable over periods 2 and 3 (6.7% and 5.2%, and 14.1% and 12.3%, respectively) but was lower in period 1 (1.5% and 7.0%).

The proportion of infants receiving birth HIV-PCR increased from 11.8% in period 1, to 34.5% in period 2 and 67.4% in period 3. Over the study period, 39.7% of babies with one or more high-risk criterion received birth EID. The association between having a high-risk criterion and receiving a birth test was strongest in period 2.

		total n=2012			period 1 n=272			period 2 n=1391			period 3 n=349		
RISK FACTOR		**n(%)**	**birth n(%)†**	**p**	**n(%)**	**birth†**	**p**	**n(%)**	**birth†**	**p**	**n(%)**	**birth†**	**p**
HIV diagnosis during pregnancy	**yes**	122 (6.1)	52(42.6)	0.02	10(3.7)	4(40)	0.012*	104(7.5)	45(43.3)	0.004	8(2.3)	3(37.5)	0.272*
	no	1890(93.9)	615(32.5)		262(96.3)	24(9.2)		1287(92.5)	383(29.8)		341(97.7)	208(61)	
Antenatal care	**none**	103 (5.1)	48(46.6)	0.003	13(4.8)	4(30.8)	0.034*	66(4.74)	35 (53.0)	<0.0001	24(6.9) ‡	9(37.5)	0.017
	≥1 visit	1909(94.9)	619(32.4)		259(95.2)	24(9.3)		1325(95.3)	393(29.7)		325(93.1)	202(65.2)	
Seroconversion during pregnancy	**yes**	31(1.5)	15(48.7)	0.069	3(1.1)	1(33.3)	0.279*	23(1.7)	12(52.2)	0.025	5(1.4)	2(40)	0.388*
	no	1981(98.5)	652(32.9)		269(98.9)	27(10.0)		1368(98.4)	416(30.4)		344(98.6)	209(60.8)	
Duration of ART	**<12weeks**	420(20.9)	191(45.5)	<0.0001	77(28.3)	20(26.0)	<0.0001	299(21.5)	147(49.12)	<0.0001	44(12.6)	24(54.6)	0.391
	>12weeks	1592(79.1)	476(29.9)		195(71.7)	8(4.1)		1092(78.5)	281(25.7)		305(87.4)	187(61.3)	
Viral load n=1346	**>1000copies/ml**	201(10.0)	105(52.2)	<0.0001	7(2.6)	2(28.6)	0.076*	145(10.4)	80(55.6)	<0.0001	49(14)	23(46.9)	0.037
	<1000 copies/ml	1811(30.0)	562(31.0)		275(97.4)	26(9.8)		1249(89.6)	348(27.9)		300(86)	188(62. 7)	
Gestational age (weeks) n=1898	**<37weeks**	115(6.1)	83(72.2)	<0.0001	4(1.6)	1(25)	0.350*	93(7.0)	68(73.1)	<0.0001	18(5.2)	14(77.8)	0.139*
	>37weeks	1783(93.9)	523(29.3)		252(98.4)	25(9.9)		1228(93)	321(26.1)		303(86.8)	177(58.4)	
Birth weight (grams) n=1928	**<2500g**	260(13.5)	159(32.9)	<0.0001	19(7.6)	3(15.8)	0.290*	196(14.6)	125(63.8)	<0.0001	45(13.4)	31(68.9)	0.174
	>2500g	1668(86.5)	476(28.5)		232(92.4)	22(9.58)		1144(85.4)	284(24.8)		292(86.7)	170(58.2)	

Table C.2. The proportion of infants per high-risk criterion who received a birth HIV-PCR per policy period.

ART – antiretroviral therapy; Viral load – Viral load recorded during pregnancy or within two weeks of delivery; Gestational age – gestational age at birth as recorded in the labour ward delivery register; Birth weight < 2500g meets the clinical definition of low birth weight.

* statistical test: Fisher Exact test; otherwise $\chi 2$ test used to assess the difference between the proportions with and without birth HIV-PCR per high-risk criterion.

† The proportion of infants with the risk factor who received birth HIV-PCR

‡ The proportion of women who received no antenatal care is slightly higher during period 3 as we continued to enrol 'unbooked' women to June 2016.

Facility of Birth

The majority of the infants (41.7%) were born at MPMOU with the numbers dropping as the level of care increased (Table 3). At all sites, the proportion of HIV-exposed infants receiving birth HIV-PCR increased with the guideline changes. Most of the birth tests were performed in hospital and not at the primary care site, even in period 3.

Facility	total n=2012		period 1 n=272		period 2 n=1391		period 3 n=349	
	n(%)	birth n(%)	n(%)	birth n(%)	n(%)	birth n(%)	n(%)	birth n(%
MPMOU	838 (41.7)	150(17.9)	126(46.3)	6(4.8)	548(39.4)	87(15.9)	164(47.0)	57(34.8)
MPDH (level 1)	710 (35.3)	301(42.4)	83(30.5)	1(1.2)	520(37.8)	207(39.8)	107(30.7)	93(86.9)
MMH (level 2)	343 (17.1)	141(41.1)	59(21.7)	20(33.9)	235(16.9)	82(34.9)	49(14.0)	39(79.6)
GSH (level 3)	98 (4.9)	61(62.2)	3(1.1)	1(33.3)	75(5.4)	46(61.3)	20(5.7)	14(70)
Other	23 (1.1)	14(60.9)	1	0	13(0.9)	6(46.4)	9(2.6)	8(88.9)
All facilities	2012	667(33.2)	272(13.5)	28(10.3)	1391(69.1)	428(30.8)	349(17.4)	211(60.5)

Table C.3. The proportion of infants with birth HIV-PCR born in each facility per policy period MPMOU – Mitchell's Plain Midwife Obstetric Unit; MPDH – Mitchell's Plain District Hospital (level 1); MMH – Mowbray Maternity Hospital (level 2); GSH – Groote Schuur Hospital (level 3); Other – MOUs and hospitals in the Western Cape outside the study facilities.

Timing of HIV-PCR and follow-up testing

1794 (89.2%) HIV-exposed infants had at least one HIV-PCR, 88.1% (1580) at 6-10 weeks (Table 4). This dropped from 92.9% of infants having HIV-PCRs at six weeks in period 1 to 80.2% in period 3. No HIV-PCR result could be found for 218 (10.8%) infants; death was confirmed in three of these. Among 656 infants who tested HIV-PCR *negative at birth*, 526 had a follow-up HIV-PCR per guideline recommendations (80.2%.)

	total n=2012	period 1 n=272	period 2 n=1391	period 3 n=349
At least one HIV-PCR n (%)	1794 (89.2)	238 (87.5)	1242 (89.3)	313 (89.7)
Single HIV-PCR only*	1149(64.0)	182(76.5)	829(66.7)	138(44.1)
Age first PCR, weeks median (IQR)	6.0 (0.1 – 6.6)	6.3 (6.0 – 6.9)	6.1 (0.1 – 6.6)	0.1 (0 – 6.1)
Birth HIV-PCR present n (%)	667 (37.2)	28 (11.8)	428(34.5)	211 (67.4)
6-10 week HIV-PCR present* n (%)	1578 (88.1)	221 (92.9)	1106 (89.8)	251 (80.2)
Follow-up HIV-PCR ever after birth test* n (%)	526 (80.2)	21 (77.8)	337 (80.8)	168 (79.6)
Age F/U HIV-PCR*, weeks median (IQR)	6.5 (6.1-8.3)	6.3 (6.1 – 6.6)	6.4 (6.0 – 7.1)	8.6 (6.4 – 10.7)
HIV-PCR positive ever n(%)	32 (1.8)	1 (0.4)	25 (2.0)	6 (1.9)
Overall transmission rate	1.8	0.4	2.0	1.9
***In utero* transmission rate (95% CI)**	1.7 (0.8-2.9)	3.6 (0.1-19)	2.3 (1.1-4.3)	0
Detection rate at 6-10 weeks* (95% CI)	0.6 (0.3-1.0)	0	0.5 (0.1-1.1)	2.3 (0.9-5.1)
Median (IQR) age HIV-PCR positive, weeks	6.4 (0.4 – 40)	0.1	6.0 (0.1 – 40.1)	6.8 (6.4 – 12)

Table C.4. Timing of HIV-PCR by policy period and HIV transmission.

*denominator excludes those with positive birth HIV-PCR

HIV transmission

HIV transmission in all infants for whom an HIV-PCR result was available was 1.8% (32/1794). This included *in utero*, intra-partum and post-partum infections (Table 4). The median age of HIV diagnosis was 6.4 weeks. The *in utero* transmission rate (i.e. proportion of infants with birth tests in whom HIV infection was detectable at birth) was 3.6% in period 1 (n=1) and 2.3% in period 2. There were no positive birth HIV-PCR results in period 3. Overall, 34.4% of all HIV infections were identified at birth – the proportion being highest in period 2 (40%). In period 3 100% of HIV infections were detected at six-weeks or later.

Of all birth HIV-PCRs (n=667), 11 (1.7%) were positive, 654 (98.0%) were negative, and two were indeterminate (0.3%). Both of these infants tested negative on repeat assays, at eight weeks and 6.1 weeks. Eleven (0.7%) infants were identified as HIV infected at the 6-10 week time-point, 1553 (98.4%) tested negative and 15 (0.9%) indeterminate. Of the latter, all but one for whom no further results could be found were negative on subsequent testing. Five of the 11 infants first identified as HIV infected at 6-10 weeks had had a negative birth HIV-PCR indicating intra-partum or early post-natal transmission.

Seven of 450 infants (negative/unknown status) who had HIV-PCR after 14 weeks old were HIV-infected giving a detection rate of 1.6% at this time-point (median age at diagnosis 51.2 weeks [IQR 32.0 – 64.1]). Two infants had negative birth and 6-10 week HIV-PCR results, four had negative 6-10 week results (no birth test) and the remaining infant was testing for the first time after 14 weeks old.

Predictors of 6-10 week testing

Maternal characteristics associated with risk of transmission were also associated with not receiving a 6-10 week HIV-PCR in both univariable and multivariable analyses. Infants who received EID at birth were 82% less likely to receive follow-up HIV-PCR than those who did not, after adjusting for policy period, low birth weight and prematurity, and maternal characteristics associated with not undergoing a 6-10 week HIV-PCR (aOR 0.18 [95% CI 0.12 – 0.26])(Table 5).

	6-10 week PCR done ever	
	Crude OR (95% CI)	**aOR (95% CI)**
Maternal age > 35y	1.47 (0.99 – 2.16)	1.32 (0.84 – 2.10)
HIV diagnosis during pregnancy	0.71 (0.41 – 1.21)	1.10 (0.56 – 2.15)
antenatal care - none	0.24 (0.15 – 0. 39)	0.25 (0.15 – 0.42)
seroconversion during pregnancy	0.24 (0.11 – 0.52)	0.33 (0.13 – 0.88)
duration of art ART<12w	0.45 (0.33 – 0.61)	0.61 (0.42 – 0.88)
viral load>1000copies/ml	0.51 (0.34 – 0.76)	0.68 (0.43 – 1.10)
gestational age <37 weeks	0.56 (0.33 – 0.95)	1.12 (0.59 – 2.11)
birth weight<2500g	0.48 (0.33 – 0.69)	0.91 (0.56 – 1.47)
EID period 2 (versus period 1) EID period 3 (versus period 1)	0.63 (0.37 – 1.10) 0.31 (0.18 – 0.55)	0.92 (0.48 – 1.75) 0.61 (0.30 – 1.47)
Birth HIV-PCR present	0.15 (0.11 – 0.20)	0.18 (0.12 – 0.26)

Table C.5. Univariate and Multivariate analysis of predictors of having a follow-up HIV-PCR as per guidelines (i.e. 4-14 weeks old) ART – antiretroviral therapy; EID – early infant diagnosis

6. Discussion

To our knowledge this is the first prospective study demonstrating the real-world implementation of the progressive introduction of birth HIV-PCR to an EID programme in a routine setting. The majority of infants received at least one HIV-PCR, most around six weeks old. As the indications for birth HIV-PCR expanded, the proportion receiving birth EID

and more than one test increased and the median age at testing decreased, but there was no change in the proportion of infants receiving at least one HIV-PCR. In addition, receipt of birth HIV-PCR significantly reduced follow-up testing. While coverage of birth HIV-PCR during period 3 was high overall (67%), it was considerably lower at the primary care facility (~35%) where >40% of deliveries occurred in this cohort. This suggests that even in a relatively well-resourced setting like the Western Cape, implementation of birth EID guidelines is challenging in primary care, the setting where most facility-based deliveries are likely to occur in sub-Saharan Africa.

Data presented in a review of the first year of the SA national programme demonstrated an increase in birth EID coverage from 39% (high-risk) to 93% within 12 months of the national guideline change with a corresponding drop in six-weeks testing (to 19%); the increase in 10-week tests was modest (13). In our study, the majority of all birth testing occurred in infants born in hospital. During the first two policy periods, this could be expected owing to the definition of some high-risk criteria (prematurity, low birth weight). This is reflected in an analysis of Western Cape laboratory data in which, while the numbers of facilities offering birth EID increased during period 2, the majority of tests continued to be performed in hospitals: 67% versus 33% sent from primary care (14). The national programme data (13) were not stratified by level of care and there are still limited data on how the EID recommendations are implemented in primary care facilities and in rural areas across SA.

Risk Factors for Vertical Transmission

Almost half of all infants presented with at least one high-risk factor for vertical transmission, yet only 30.8% received birth HIV-PCR during period 2, the majority of those being premature and low birth weight infants (i.e. likely to be born in hospital). Although the likelihood of receiving a birth test increased with increasing number of risk factors, many infants at high risk were missed.

Nevertheless, these data demonstrate the maturation of the HIV treatment programme in Cape Town and some progress towards addressing vertical transmission risk factors: over 28 months an increasing number of women entered antenatal care known to be HIV-infected, had conceived on ART and were on ART for longer durations before delivery.

HIV Transmission and Follow-up Testing

The number of HIV-infected infants in the cohort was low so data need to be interpreted with caution. Overall vertical HIV transmission was 1.6%. This compares with a national rate of 1.4% at six-weeks in 2016 down from 2.4% in 2012 (15, 16). *In utero* transmission was 1.7% on average, highest during periods 1 (3.6%) and 2 (2.3%) when a sample of high-risk infants was selected. A hospital-based retrospective cohort study in Cape Town found a similar *in utero* transmission rate of 3.8% under a programme of targeted birth EID (17). When the sample of exposed infants tested at birth is expanded to include those at low risk of infection, a reduced transmission rate would be expected. The average *in utero* transmission rate during the first year of the national birth EID programme was 1.1%; when stratified by province it was 2.6% in the Western Cape but the interval overlapped both period 2 and period 3 suggesting that the sample may have been biased towards high-risk infants and is closer to that in period 2 (13). The discrepancy may also be due to the maternal HIV prevalence in our study sample (14.7% at delivery) versus 18.9% for the whole province (18). In a Johannesburg hospital sample *in utero* transmission rate was 1.4% during period 3 (19).

The detection rate between birth and 6-10 weeks was lower than at birth except in period 3; this compares with rates of 0.4 – 0.5% in a hospital cohort during the same period of targeted birth testing (17). During period 3 there were no positive birth HIV-PCR results and the detection rate from birth to 6-10 weeks was 2.4% (versus national rates of 1.4% at six weeks (13).) Almost half of these infants had a negative birth test indicating late intra-partum or early post-natal transmission and reinforcing the necessity of follow-up testing.

Our study provides additional evidence that receipt of birth HIV-PCR decreased the presentation for follow-up testing (13, 14, 17). Receipt of birth EID reduced the likelihood of subsequent HIV testing after six-weeks by 82%, even when controlling for other risk factors. This concurs with a retrospective analysis of a Cape Town hospital sample in which infants who had birth HIV-PCR were 40% less likely to present for follow-up testing (17); when follow-up did occur in the birth cohort it was at older ages than among those who did not have birth EID (8.6 versus 7.1 weeks). We found that age of subsequent HIV-PCR was greatest during the period of maximum birth testing, i.e. 8.6 weeks in period 3; appropriate

as the time-point for testing had shifted from six to 10 weeks. In a sensitivity analysis to assess the influence of birth EID on the likelihood of *ever* having a repeat HIV-PCR after four weeks old (i.e. not restricted to the 6-10 week window), the direction of the associations remained unchanged. These findings are concerning given that the modelling data that supported the introduction of birth EID in SA demonstrated a loss of effectiveness if return for follow-up testing dropped below 63% (5). In addition, at least 11 HIV infected infants in this cohort (35.5%) were late intra-partum or postnatally infected, emphasising the need for testing after the birth time-point. In contrast to the SA data, interim analysis of women in Lesotho indicated that the majority of women who received very early EID (within two weeks of birth) returned for results and follow-up testing at six-weeks(20, 21).

The proportion of indeterminate results was low at all time-points, but accounted for 15.4% and 55.6% of non-negative results at birth and 6-10 weeks respectively. Similar results were reported in a review of birth EID in Johannesburg, 0.4% of all results, 24% of non-negative results were indeterminate (22). In the Johannesburg study, additional resources were required to trace and retest these neonates. In our cohort all the infants were negative on repeat testing, which is in contrast to both the Johannesburg study and to a laboratory-based cohort which demonstrated increased likelihood that infants with indeterminate HIV-PCR result would be positive on subsequent testing in a setting of intensified VTP regimens (22, 23). It is possible that the repeat negative HIV-PCR results in our report were false negatives in the face of prolonged infant post-exposure prophylaxis and exposure to ART in breast-milk.

Strengths and limitations

We present prospective individual longitudinal follow-up on a cohort of HIV-exposed infants born at a primary care facility as well as in hospital. The cohort has an advantage over the aggregate laboratory data (13, 14) in that HIV-PCR results could be attributed to individual linked mother-infant pairs. One of the acknowledged challenges of using routine laboratory data for surveillance in SA is the lack of an unique patient identifier and the inability to accurately de-duplicate data (13, 15). Although the Western Cape has made substantial progress with issuing unique identifiers to infants at birth, operational challenges remain, and our sample was limited to linked mother-infant pairs, compromising numbers that could

be included, especially in period 1. While our study is strengthened by demonstrating real-world implementation of expanded EID guidelines, it is consequently also dependent on the quality of routine clinical sources and we were unable to account for missing data. It is possible that not all the relevant HIV-PCRs were recorded as an infant could have numerous alternative identifiers; we were also unable to determine whether HIV-PCR tests occurred outside the Western Cape. Some infants died before follow-up testing. Prematurity and low birth weight contribute to infant mortality independent of HIV infection. Similarly infants may have been too acutely unwell to undergo birth EID.

7. Conclusions

In this study we assessed the impact of different birth EID strategies, that of targeted birth testing of high-risk infants, and universal testing of all HIV-exposed infants. Targeted birth EID identified more HIV-infected infants early, some of whom may otherwise have died before six-weeks. However, universal testing simplified the VTP guidelines and increased the proportion of infants tested at birth. While our data demonstrate an encouraging response to EID guideline changes, birth HIV-PCR was not well implemented at primary care level. Birth EID also compromises follow-up testing at six or 10 weeks and this LTFU may negate the model-predicted benefits of birth HIV-PCR (5). Additional intervention is required to reduce risk factors for transmission, to expand birth EID at primary care and to improve follow-up testing at the 10 week time-point in SA.

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Appendix 1: Closing the Gaps

Towards pediatric HIV elimination: Closing the gaps in prevention of mother-to-child transmission (PMTCT) programme coverage, early infant diagnosis and treatment.
(Short title: "Closing the Gaps")

1. Background and project outline

Virtual elimination of vertical transmission of HIV is within reach in South Africa. Despite high antenatal HIV prevalence, implementation of currently available technology and guidelines in the South African Prevention of Mother-to-Child Transmission (PMTCT) programme has resulted in substantial reductions in vertically transmitted HIV to 3.5% across the country, and <2% in the Western Cape (WC). Yet, elimination remains elusive due to persistent coverage gaps and "drop-offs" at each of the steps required in completion of the PMTCT and infant care continuum, with upwards of 1000 vertically infected infants born in the Western Cape each year. We hypothesize that these persistent coverage gaps are the most important remaining barrier to near-elimination of pediatric HIV morbidity and mortality, irrespective of intensification of PMTCT regimens.

There is thus a need for research to identify strategies that provide an early warning system of coverage gaps both at the level of the health system and the individual, and link to care pregnant women and HIV-exposed/infected infants with such gaps who critically need antiretroviral therapy (ART) or interventions to prevent transmission.

2. Purpose and objectives:

The purpose of this study is to implement and evaluate three linked active surveillance activities integrated with the existing service platform that aim to iteratively identify and close PMTCT, early infant diagnosis and ART coverage gaps. The study will be carried out by the Centre for Infectious Disease Epidemiology and Research (CIDER) from the UCT School of Public Health and Family Medicine in partnership with the WC Department of Health (DoH). The study will be conducted at the Gugulethu Midwife Obstetric Unit (GMOU) in the Klipfontein sub-district, and its referral facilities, Mowbray Maternity Hospital (MMH) and Groote Schuur Hospital (GSH).
The three activities are as follows:

1. Routine cord blood surveillance (CBS) for HIV exposure and, if positive, the presence of antiretroviral drugs (ARVs): This will be linked to the existing programme of screening for congenital hypothyroidism. CBS will enable identification and urgent linking to care of HIV-exposed infants with no/suboptimal peripartum ARVs to ensure interventions to prevent postnatal transmission, prompt infant diagnosis and infant ART if infected.

2. A single integrated electronic PMTCT register (e-register) linking existing separate paper-based registers held at antenatal, obstetric and infant clinics in the Klipfontein subsdistrict, as well as NHLS laboratory data (maternal CD4, infant PCR). Unlike existing registers that report on aggregate coverage of individual steps in the PMTCT pathway, the combined e-register will track an individual pregnant woman's trajectory through PMTCT and identify where drop-offs and missed opportunities occur. The e-register will therefore support systems of urgent reporting of laboratory results of low CD4 counts in

pregnant women and positive infant HIV-PCR test results to clinics, with tracing to ensure prompt ART initiation.

3. Clinical quality assurance and improvement using CBS and PMTCT e-register data. This will be developed in partnership with DoH service managers and integrated with existing subdistrict programme review processes.

These activities thus aim to close coverage gaps at 2 levels:

At an individual level: For individual mothers and infants interventions are designed to ensure appropriate linkages to care will be triggered at key points in the PMTCT cascade.
At a health system level: An auditable early warning system of coverage gaps across the entire program, and interrogation of the reasons for ongoing vertical transmission, will inform and be integrated into clinical quality assurance systems.

Objectives of the study are therefore as follows:
1. To compare PMTCT programme outcomes and effectiveness before and after implementation of these surveillance activities. Measures of programme outcomes and effectiveness to be compared will include:

- Vertical transmission proportion
- Proportion of women presenting at delivery without a previous HIV test
- Proportion of women with incident HIV infection during pregnancy
- Proportion of mother-infant pairs "dropping off" at each step of the cascade (e-register and CBS).

2. To assess the feasibility and success of implementation of the project by measuring, for example, CBS coverage, completeness and quality of PMTCT e-register data entry and linkage, proportion of those identified by the e-register and/or CBS as needing additional intervention who are successfully traced and linked to appropriate care, compliance with clinical quality assurance review meetings.

All data needed for the above objectives will be collected through implementation of CBS and the e-register.

3. Implementation and recruitment
Briefly we aim to implement the 3 activities as follows:

3.1. Cord blood surveillance
The project will build on the existing system of congenital hypothyroidism screening, using routine systems for collection of a specimen of cord blood, specimen transport, registration and testing. A research assistant will visit GMOU, MMH and GSH twice a week to support and monitor the collection of cord-blood specimens. Recruitment of pregnant women ≥18 years of age will take place at the antenatal booking visit at GMOU. As part of counselling and testing for routine opt-out antenatal HIV testing that is standard of care, a study counsellor will work with existing counselling staff to obtain informed consent (IC) to participate in the study. This will include consent for a rapid HIV test to be performed on

a cord blood specimen taken at delivery and, if HIV positive, 2 dried blood spot specimens to be tested for the presence of ARVs. HIV tests will be performed at the NHLS laboratory at Red Cross Children's Hopsital and tests for ARVs at the UCT Department of Pharmacology. The process of obtaining IC will include explaining the risks and benefits of participation (including that testing may lead to more active tracing and follow-up of the patient if results indicate that there is a high risk of transmission), the alternatives to participation, and that the decision to participate will in no way affect the medical care provided. Patients consenting to participate in the study will be provided with an information sheet and informed that they may withdraw from the study at any time. In particular, at delivery, women will be reminded that they have given consent for cord blood testing, and given the opportunity to withdraw from the study. For women who arrive at GMOU in labour without a previous antenatal booking, we will attempt to obtain consent during labour to participate in the study. This will be done to ensure that coverage of CBS is as complete as possible, and that "unbooked" women are also able to access the benefits of study participation. All consent procedures, information sheets and reminders of study involvement will be performed/translated into all local languages.

Infants at high risk of HIV infection (cord blood HIV positive, no/sub-optimal ARVs) will be traced and linked to care by a study community outreach worker working with existing health service PMTCT support staff. This will include counseling regarding the HIV diagnosis to the mother if this was previously unknown. Mother-infant pairs will be linked to routine PMTCT/MCH services so that interventions to reduce the risk of postnatal transmission as well as early infant PCR testing (with early ART if positive) can be provided. Data from HIV-exposed infants with ARVs present in cord blood will be linked to laboratory data to determine whether PCR testing occurs timeously. The same tracing staff will seek infants not tested by 8 weeks of age and link them to care.

2. Electronic PMTCT Register

PMTCT data from women delivering at GMOU as well as women referred from GMOU to MMH and GSH, will be linked with data from the antenatal and infant follow-up clinics associated with GMOU, as well as with laboratory data to establish a single PMTCT e-register. Linkage fields will include the WC unique patient health numbers, maternal and infant names, birth dates, delivery dates and infant birth dates. A very high proportion of true matches are readily linkable when the linkage sets are restricted by location (facility) or date (e.g. birth date of infant and delivery date of mother). The proportion of records that cannot be linked will indicate the disjunction in each step in the PMTCT continuum, providing early warning of coverage gaps.

The e-register will support urgent notification of clinics of laboratory results of maternal CD4 <350 cells/µl and positive infant PCR tests. These will be identified through daily updates of laboratory results from the NHLS to the research officer at CIDER for incorporation into the e-register. The research officer will work with existing reporting systems to inform the relevant clinic/health service staff of any positive test results. We will work with existing WC and City of Cape Town HIV/AIDS and PMTCT to ensure tracing and treatment initiation for these HIV-infected women and infants needing ART.

There will be no recruitment and we are requesting a waiver of informed consent for this part of the project. We will only be incorporating data already collected as part of routine care by the health

services and for which informed consent was given when women consented to standard of care routine antenatal opt-out HIV testing.

3. Clinical quality assurance and improvement

Monthly program reviews will be implemented in partnership with Department of Health service managers and be informed by data arising from the PMTCT e-register and CBS. For example:

- Sentinel events of new cases of infant HIV identified through the e-register will be reviewed to identify reasons for transmission.
- Coverage gaps at each step of the PMTCT cascade in women on the PMTCT program as identified through the e-register will be reviewed.
- Coverage gaps in all steps of the cascade up to and including delivery as measured by CBS data will be reviewed.

No maternal or infant identifiers will be used or mentioned at all in these reviews.

4. Description of risks and benefits:

4.1. Potential risks to subjects and their likelihood and seriousness

The major risk for all 3 components will be breach of confidentiality - project staff will have access to individually identifiable maternal and infant HIV and ARV cord blood results, and trace high risk transmission mother-infant pairs. A small number of women will be recruited for CBS in labour and this may pose a psychological risk. Patient autonomy may also be compromised and privacy breached, as the circumstances in labour may not provide adequate opportunity for counseling and informed consent according to service protocol. This could be considered morally controversial. However, it is argued that withholding the right to consent to HIV testing and treatment to a woman of unknown serostatus compromises her autonomy to have the choice of her infant receiving interventions to prevent vertical transmission if she is HIV infected, even if consent needs to be sought during labour. The study offers clear benefits to mother-infant pairs identified as HIV infected for the first-time at delivery (opportunity to receive proven effective interventions to prevent post-natal transmission, active follow-up and linkage to HIV/ART care) so it would be unethical to exclude women presenting for the first time when already in labour. This is the group for whom the benefits of participation may be the greatest. Rapid HIV testing in labour is recommended in other service settings e.g. “opt out” testing in labour for a woman of unknown serostatus is standard of care in the United States.

4.2. Adequacy of Protection Against Risks

The process of obtaining informed consent for CBS in such a way as to minimize risks, ensure that women fully understand the risks of participation and have autonomy to refuse participation or withdraw from the study have been described above. In particular, we will only use qualified HIV Counseling and Testing (HCT) counselors and will employ additional counselors to support DoH counselors to ensure adequate quality of the informed consent process. For women recruited during labour we will ensure that counseling is conducted in privacy and that the benefits and risks of cord blood HIV testing are fully explained. Since a pregnant woman in labour is particularly vulnerable, we will be sure that women clearly understand that the quality of health care they receive will not be

compromised should they elect not to participate. Should a woman with no prior antenatal care present too late in labour for there to be time for her to be invited to participate in the study, the midwife will collect the additional cord blood specimen, and after delivery is complete, the woman will be invited to participate in the study and have the specimen tested for HIV/ARVs. Specimens from women who decline to participate post-delivery will be discarded.

Tracing and linkage to care of pregnant women eligible for ART and infants identified as HIV-infected or at high risk of vertical transmission will be done carefully and sensitively. Pregnant women/mothers of infants will be telephoned and asked to attend the clinic urgently for a health care appointment. Where telephonic tracing is unsuccessful, a home visit will be done and the patient asked to attend the clinic for a health care appointment. No laboratory results will be given to the mother telephonically or at the home visit. Results will only be given to the mother at the clinic appointment in a private room by qualified health service providers as per South African PMTCT and HCT guidelines ensuring patient confidentiality. Full post-test counselling will be given to the mother according to HCT guidelines.

All HIV counselling and testing (antenatally and during labour or post-delivery) will be offered by qualified health service providers as per HCT and PMTCT guidelines ensuring patient confidentiality and autonomy.

To minimize risks of breaching confidentiality, all electronic project data will be stored in a password-protected database on a secure server housed in the CIDER offices at the UCT Faculty of Health Sciences, with user level access control implemented. Any paper based patient records will be stored in locked filing cabinets in locked room with access only to those directly involved in the study. Inclusion of identifiers in the databases is needed initially so that they can be linked and for tracing of mothers/infants. Identifiers will be removed from all databases (PMTCT e-register and CBS HIV and ARV results) as soon as possible once linkage and tracing are complete. Only staff directly involved in the project will have access to the data and will undergo Human Subjects Protection training prior to having access to any individually identifiable data. We will use the same measures to protect against breach of confidentiality as are currently in place in all CIDER projects. Thus far, no breach of confidentiality has occurred in any of these projects. Should a breach of confidentiality occur in this project, it will be noted and investigated.

4.3 Potential benefits

Historically, antenatal sentinel surveillance and monitoring of PMTCT programmes has been based on anonymous specimens and aggregate data. While these provide surveillance for the programme as a whole, they provide no opportunity to act on clinically important results, and thus fail to benefit the individual patient on whom testing has been performed.

This study thus has the following benefits:

- It will generate surveillance data and early warning systems that will trigger and inform PMTCT programme improvement, thus benefiting all HIV-infected mother-infants pairs in the area where the study takes place.

- It provides individual benefit to mothers and infants participating in the study by identifying pregnant women eligible for ART, HIV-infected infants and those at high risk of transmission and prioritizing provision of immediate PMTCT and maternal and infant HIV care interventions that are known to be effective and safe. All pregnant women ≥18 years of age will be invited to join the study and have access to these benefits.
- The surveillance systems will be developed in partnership with health services with a long term view to more widespread routine adoption, so that HIV-infected women and exposed infants from other areas will also benefit if the project is successfully expanded to other areas and integrated into routine surveillance.
- The findings of the study will inform PMTCT programmes broadly in terms of improving coverage and maximizing the benefits of ART for maternal and child health.

4.4. Balancing the major risks to subjects with the potential benefits of the project

***Breach of confidentiality*:** As every effort will be taken to ensure confidentiality, the small risk of it being breached can be considered to be acceptable given the potential not only to achieve PMTCT programmatic improvements, but also to provide individual benefit to study participants through tracing and linkage to care. Of note, the individual benefits could not be provided without the holding of identified laboratory test results, so this is an unavoidable risk.

Compromise of patient autonomy and psychological distress for women recruited during labour:

While optimal strategies for PMTCT comprise early antenatal diagnosis and treatment, testing pregnant women of either unknown serostatus or with acute infection at the time of delivery provides an additional opportunity for PMTCT. Given the availability of effective maternal and infant ART the potential immediate benefit to mother and child of a pregnant woman learning her serostatus peripartum has increased relative to the potential risks of offering HIV testing in this setting.

Appendix 2: Western Cape Vertical Transmission Prevention of HIV and Early Infant Diagnosis Algorithms

Western Cape Government Health
BETTER TOGETHER.

ALGORITHM 1: PMTCT

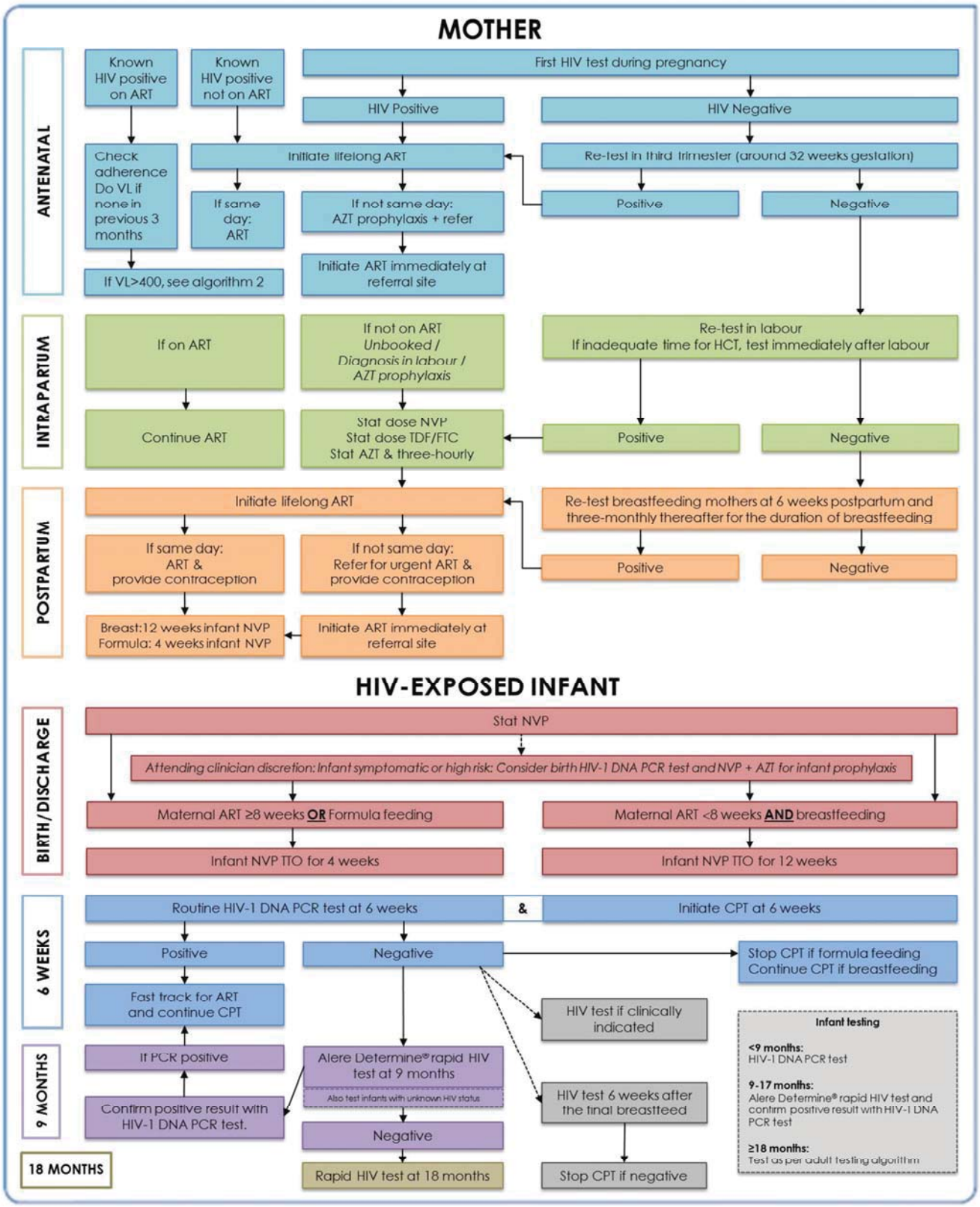


Figure 1. Algorithm 1: PMTCT

Appendix 2: Western Cape Vertical Transmission Prevention of HIV and Early Infant Diagnosis Algorithms

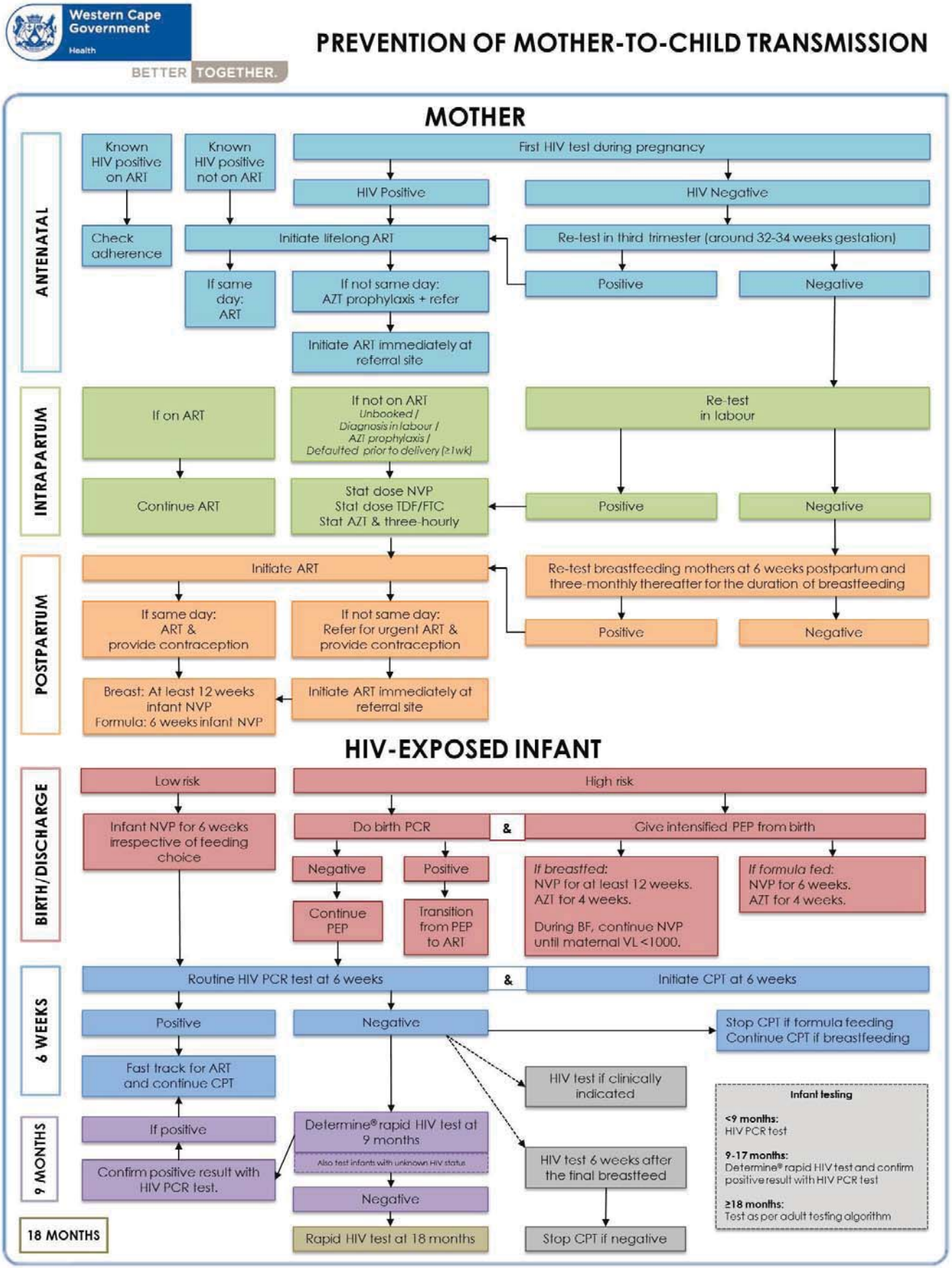
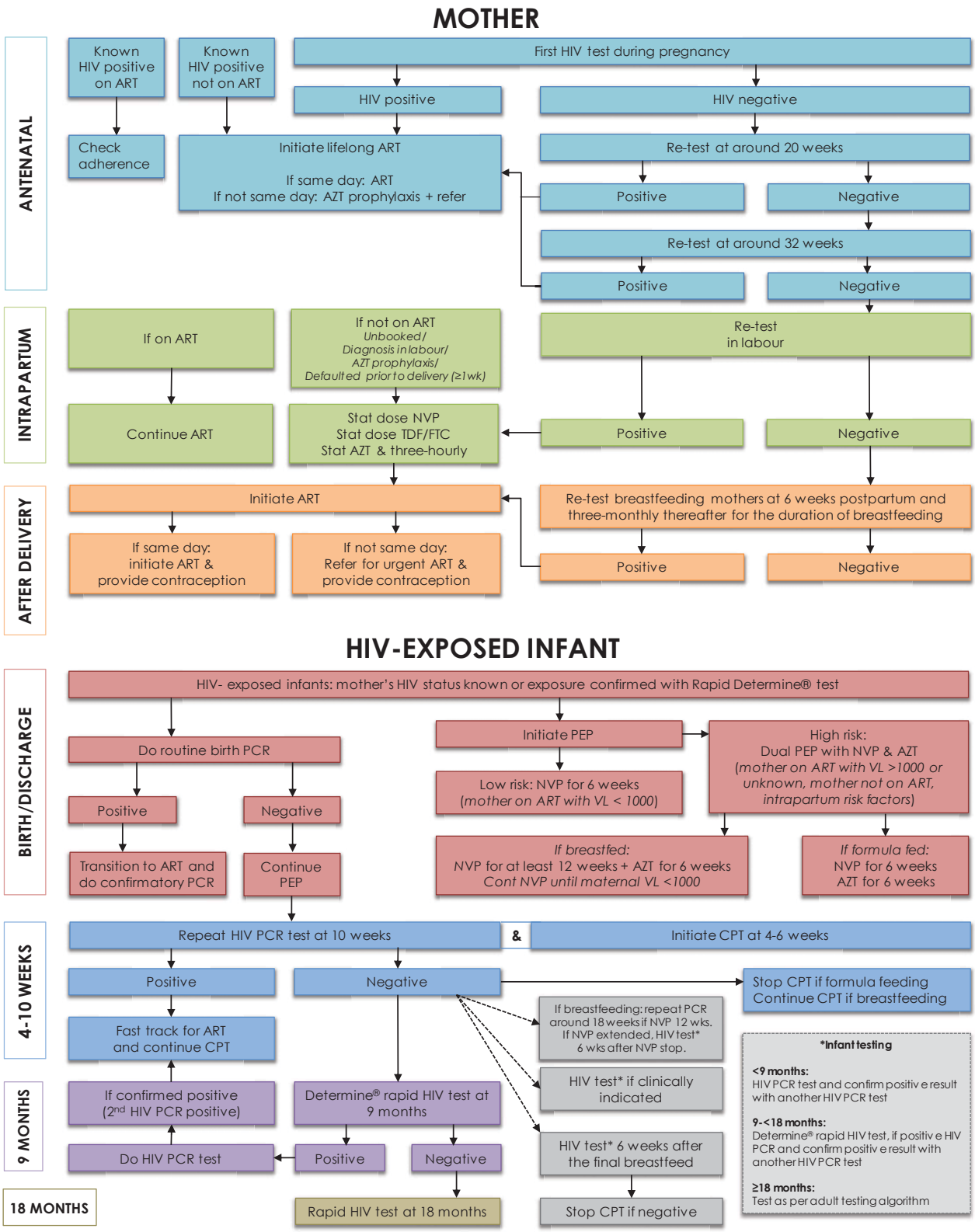


Figure 1: Algorithm: Prevention of Mother-to-Child Transmission

Appendix 2: Western Cape Vertical Transmission Prevention of HIV and Early Infant Diagnosis Algorithms

Annexure 1: PMTCT Algorithm



Appendix 3: UCT Human Research Ethics Commitee approval (145/2013) & PGWC Research Approval (RP063/2013)

UNIVERSITY OF CAPE TOWN

Faculty of Health Sciences
Faculty of Health Sciences Human Research Ethics Committee
Room E52-24 Groote Schuur Hospital Old Main Building
Observatory 7925
Telephone [021] 406 6338 • Facsimile [021] 406 6411
e-mail: sumayah.ariefdien@uct.ac.za
www.health.uct.ac.za/research/humanethics/forms

29 April 2013

HREC REF: 145/2013

Dear A/Prof A Boulle
CIDER
School of Public Health & Family Medicine
FHS

Dear A/Prof A Boulle

PROJECT TITLE: CLOSING THE GAPS IN PMTCT PROGRAM COVERAGE, EARLY INFANT DIAGNOSIS AND TREATMENT.

Thank you for addressing the issues raised by the Human Research Ethics Committee.

It is a pleasure to inform you that the HREC has **formally approved** the above mentioned study.

Approval is granted for one year till the 15 May 2014.

Please submit a progress form, using the standardised Annual Report Form, if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please quote the REC. REF in all your correspondence.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, HSF HUMAN ETHICS

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB00001938

sAriefdien

Western Cape Government
Health

STRATEGY & HEALTH SUPPORT
Health.Research@westerncape.gov.za
tel: +27 21 483 6857; fax: +27 21 483 9895
5th Floor, Norton Rose House,, 8 Riebeek Street, Cape Town, 8001
www.capegateway.gov.za)

REFERENCE: RP 063 /2013
ENQUIRIES: Ms Charlene Roderick

Centre for Infectious Disease Epidemiology and Research
Falmouth Building
Faculty of Health Sciences
University of Cape Town
Observatory
7925

For attention: **Dr MA Davies, Dr EM Kalk, Prof J McIntyre, Prof A Boulle, Prof D Coetzee and Dr M Kroon**

Re: Towards paediatric HIV elimination: Closing the gaps in prevention of mother-to-child transmission (PMTCT)programme coverage, early infant diagnosis and treatment"Closing the Gaps"

Thank you for submitting your proposal to undertake the above-mentioned study. We are pleased to inform you that the department has granted you approval for your research.
Please contact the following people to assist you with any further enquiries in accessing the following sites:

Mitchells Plain Hospital **Ms Z Xapile** **Contact No. 021 391 5820**

Kindly ensure that the following are adhered to:

1. Arrangements can be made with managers, providing that normal activities at requested facilities are not interrupted.
2. Researchers, in accessing provincial health facilities, are expressing consent to provide the department with an electronic copy of the final report within six months of completion of research. This can be submitted to the provincial Research Co-ordinator (Health.Research@westerncape.gov.za).
3. The reference number above should be quoted in all future correspondence.

Yours sincerely

DR NT Naledi
DIRECTOR: HEALTH IMPACT ASSESSMENT
DATE: 11/11/2013
CC **P OLCKERS** **DIRECTOR: MITCHELLS PLAIN / KLIPFONTEIN**

Appendix 4: UCT Human Research Ethics Committee approval (097/2018)

UNIVERSITY OF CAPE TOWN
Faculty of Health Sciences
Human Research Ethics Committee

Room E53-46 Old Main Building
Groote Schuur Hospital
Observatory 7925
Telephone [021] 406 6492
Email: sumayah.ariefdien@uct.ac.za
Website: www.health.uct.ac.za/fhs/research/humanethics/forms

08 February 2018

HREC REF: 097/2018

A/Prof M Davies
CIDER
SPHFM-5th Floor
Falmouth Building-FHS

Dear A/Prof Davies

PROJECT TITLE: A LONGITUDINAL ANALYSIS OF NEONATAL AND INFANT DIAGNOSTIC HIV PCR UPTAKE AND ASSOCIATIONS DURING THREE SEQUENTIAL POLICY PERIODS IN MITCHELL'S PLAIN CAPE TOWN (Master's Dr E Kalk) sub-study-145/2013

Thank you for submitting your study to the Faculty of Health Sciences Human Research Ethics Committee (HREC) for review.

It is a pleasure to inform you that the HREC has **formally approved** the above-mentioned study.

Approval is granted for one year until the 28 February 2019.

Please submit a progress form, using the standardised Annual Report Form if the study continues beyond the approval period. Please submit a Standard Closure form if the study is completed within the approval period.
(Forms can be found on our website: www.health.uct.ac.za/fhs/research/humanethics/forms)

We acknowledge that the student: Dr E Kalk will also be involved in this study.

Please quote the HREC REF in all your correspondence.

Please note that the ongoing ethical conduct of the study remains the responsibility of the principal investigator.

Please note that for all studies approved by the HREC, the principal investigator **must** obtain appropriate institutional approval, where necessary, before the research may occur.

Yours sincerely

PROFESSOR M BLOCKMAN
CHAIRPERSON, FHS HUMAN RESEARCH ETHICS COMMITTEE

Federal Wide Assurance Number: FWA00001637.
Institutional Review Board (IRB) number: IRB0001938

APPENDIX 5: Instructions to authors:
Journal of the International AIDS Society

Sections

1. SUBMISSION

Please carefully read through the Instructions for Authors and prepare your manuscript according to the guidelines, including structuring it manuscript based on the chosen article category. Manuscripts that do not follow the instructions may be returned to the authors for corrections.

Authors should kindly note that submission implies that the content has not been published or submitted for publication elsewhere except as a brief abstract in the proceedings of a scientific meeting or symposium.

Once the submission materials have been prepared in accordance with the Author Guidelines, manuscripts should be submitted online at https://mc.manuscriptcentral.com/jias. The submission system will prompt authors to use an ORCID iD (a unique author identifier) to help distinguish their work from that of other researchers. Click here to find out more.

You will be asked to suggest potential peer reviewers for your manuscript: they should be experts in the field and be able to provide an objective assessment of the manuscript. Any suggested peer reviewers should not have published with any of the authors of the manuscript within the past five years, should not be current collaborators, and should not be members of the same institution. Suggested reviewers will be considered alongside potential reviewers identified by the Editorial team.

Click here for more details on how to use ScholarOne.

2. AIMS AND SCOPE

The *JIAS* welcomes submissions on HIV-related topics from across all scientific disciplines, including but not limited to:

- Basic and biomedical sciences
- Behavioural sciences
- Epidemiology
- Clinical sciences
- Health economics and health policy
- Operations research and implementation sciences
- Social sciences and humanities, including political sciences and media

The *JIAS* prioritizes submissions from operational research and implementation science as publication of such material can provide valuable information on various algorithms for monitoring and providing support for comprehensive, yet affordable and sustainable treatment, prevention and care programmes in different contexts.

Submission of HIV research carried out in low- and middle-income countries is strongly

encouraged.

3. MANUSCRIPT CATEGORIES AND REQUIREMENTS

The *JIAS* accepts submissions in the following categories:

- Research
- Short report
- Review
- Debate
- Commentary
- Letter to the Editor
- Viewpoint

Research - full reports of data from original research studies

Headings: Introduction, Methods, Results, Conclusions
Word limit: 350 words

Main text:
Headings: Introduction, Methods, Results, Discussion, Conclusions
Word limit: 3500 words
Numbers of figures and tables: Unlimited
Additional files: Yes

4. PREPARING THE SUBMISSION

Cover letter
In the cover letter, please explain why your manuscript should be published in the journal. If necessary, address any issues relating to our editorial policies .and declare any competing interests (see Editorial Policies and Ethical Considerations)

Parts of the Manuscript
The manuscript should be submitted as a main text file including figures and appendices and supporting information should be supplied as separate files.

Main Text File
The text file should be presented in the following order:

1. Title page;
2. Keywords;
3. Abstract;
4. Main text;
5. Conflict of Interest Statement;
6. Authorship;
7. Acknowledgments;
8. References;
9. Tables;
10. Figures;

Title page

The title should not contain abbreviations, except commonly used abbreviations such as HIV or AIDS (see Wiley's best practice SEO tips).

On the title page, you should mention the title of the manuscript, list all authors' names in full, and list any study groups if applicable. Each authors' affiliation should be numbered in superscript consecutively and listed underneath, including department, institution, city and country.

The corresponding author should be marked with the symbol § in superscript and full contact details should be provided, including a telephone number with country code. Authors who have contributed equally to the work should be marked with the symbol * in superscript. Deceased authors should be marked with the symbol ^ in superscript. The email addresses of all authors should be listed by their initials.

Keywords
Pease provide six keywords. Keywords should be taken from those recommended by the US National Library of Medicine's Medical Subject Headings (MeSH) browser list at https://www.nlm.nih.gov/mesh/. Preferably alternate words to those found in the abstract in order to improve search hits for the article in repositories.

Abstract
The Abstract should not exceed 350 words and should be structured according to the headings of the selected article category (see above), excluding the heading “Discussion” for Research articles. Avoid using abbreviations and do not cite references in the Abstract. If you are reporting results from a controlled health care intervention, please include your trial registry, together with your unique identifying number at the end of the Abstract. For randomized controlled trials, follow the CONSORT extension for abstracts .

Main Text

Article sections

Introduction
The Introduction section should introduce the topic to readers without specialist knowledge in that area and must clearly outline the current state of knowledge in this field, the motivation and the aim of the study or the article.

Methods
The Methods section should include all information necessary to repeat the study, in particular, the study design, how data was collected and analyzed, clarifying the choice of methods that were made. If applicable, you should describe the setting of the study, the dates the study were conducted, and the sample or participants, as well as necessary power calculations and materials, including statistical packages, used. Interventions and programmes should be described in detail. Generic names for drugs or any molecules should be used.

All studies involving humans or animals require a statement on ethical approval, and for the former, the consent procedure that was followed. Please include the names of the ethics review board(s) that approved the study. If the research study was specific to one sex/gender, the reasons for this should be clearly stated.

Results
This section should include only data and findings from the authors' study. Presentation of statistical results should mention confidence intervals and levels of significance where appropriate. Quotes from qualitative study participants of less than three lines should be quoted in the text using quotation marks. For quotes longer than three lines, place the quote in a separate, indented paragraph and introduce it with a colon. No quotation marks are needed in this case. Details of the participant can be added in round brackets following the quote, but should not contain identifiable information to ensure confidentiality. Clarifications within the quotation should be placed in square brackets.

Submitting authors are strongly encouraged to include data disaggregated by sex (and, whenever possible, by race) and provide a comprehensive analysis of gender and racial differences. The authors should include the number and percentage of men, women and, if appropriate, transgender persons who participated in the research study. Anatomical and physiological differences between men and women (height, weight, body fat-to-muscle ratios, cell counts, hormonal cycles, etc.), as well as social and cultural variables (socio-economic, education, access to care, etc.), should be taken into consideration in the presentation of data and/or analysis of the results.

Discussion
In the Discussion section, you should discuss your main findings and place these within the context of the current body of knowledge in the field. Limitations of the study, for example, selection bias, can also be discussed, and should address how these influence the results and

conclusions. If statistically significant differences were found between men and women or between different racial or cultural groups in the effects of the studied intervention, the implications, if any, for clinical and/or public health should be adequately discussed.

Conclusions
In your Conclusions section, state your key messages from the study and explain their importance and relevance, as well as implications. Future studies and recommendations can be included in this section. The conclusions drawn must be strictly based on the data provided.

Conflict of Interest Statement
Authors will be asked to provide a conflict of interest statement during the submission process. For details on what to include in this section, see the 'Conflict of Interest' section in the Editorial Policies and Ethical Considerations section below. Submitting authors should ensure they liaise with all co-authors to confirm agreement with the final statement.

Authorship
Please refer to the journal's Authorship policy in the Editorial Policies and Ethical Considerationssection for details on author listing eligibility. The individual contributions of each author must be specified in the Authors' Contributions section. Please use authors' initials and state that all authors have read and approved the final manuscript. An example of a suitable statement is: "S.W., N.J., D.W. and S.S. performed the research. S.W., N.J., H.H. and T.L. designed the research study. H.H. and S.S. contributed essential reagents or tools. S.W., N.J. and D.W. analysed the data. S.W. and N.J. wrote the paper." Please see the 'Authorship' section in the Editorial Policies and Ethical Considerations section below for what constitutes authorship.

Acknowledgments
Contributions from anyone who does not meet the criteria for authorship should be listed, with permission from the contributor, in an Acknowledgments section. Financial and material support should also be mentioned. Thanks to anonymous reviewers are not appropriate.

References
All external sources of information should be referenced within the text, the tables and figures, using consecutive numbering in square brackets, e.g. [1], [3-5], [3,4]. The references should be up to date and adequately reflect the current state of knowledge in the field. Citation bias, for example, by country or point of view must be avoided. Numbers of references are unlimited for all article categories and should be formatted in standard Vancouver style; see Sample references from ICMJE . Unpublished observations, personal communications and manuscripts currently under consideration should be cited in the text in round brackets and not in the reference list.

Tables
They should be supplied as editable files, not pasted as images. Tables should be inserted into the text. They should have the header: "Table 1. Title of table". All tables should be cited in the text in consecutive order. The tables should not contain colour or shading, and no vertical, visible lines. If tables are copied or adapted from another source, permission must be sought by the authors prior to publication and these should be clearly cited as such. If a table spans more than one page, authors may want to consider uploading the table as an additional file instead. Tables should be self-contained and complement, not duplicate, information contained in the text. A legend can be provided underneath the title, listing any abbreviations or meanings of symbols used. If several tables are included, please ensure that symbols are used consistently. Legends should be concise but comprehensive – the table, legend, and footnotes must be understandable without reference to the text. All abbreviations must be defined in footnotes. Footnote symbols: †, ‡, §, ¶, should be used (in that order) and *, **, *** should be reserved for P-values. Statistical measures such as SD or SEM should be identified in the headings.

Figures
Figures should be cropped as closely as possible and have the header: "Figure 1. Title of figure". All figures need to be cited in the text in consecutive order.

Although authors are encouraged to send the highest-quality figures possible, for peer-review purposes, a wide variety of formats, sizes, and resolutions are accepted. Click here for the basic

figure requirements for figures submitted with manuscripts for initial peer review, as well as the more detailed post-acceptance figure requirements.

Figure legends should be concise but comprehensive – the figure and its legend must be understandable without reference to the text. Include definitions of any symbols used and define/explain all abbreviations and units of measurement. If several figures are included, please ensure that symbols are used consistently.

Additional Files

Appendices
Appendices will be published after the references. For submission, they should be supplied as separate files but referred to in the text.

Supporting Information
Supporting information is information that is not essential to the article, but provides greater depth and background. It is hosted online and appears without editing or typesetting. It may include tables, figures, videos, datasets, etc. Click here for Wiley's FAQs on supporting information.

Note : if data, scripts, or other artefacts used to generate the analyses presented in the paper are available via a publicly available data repository, authors should include a reference to the location of the material within their paper.

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The following points provide general advice on formatting and style:

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• **Acronyms:** Acronyms should be used sparingly, and not in headings or in the Abstract. Only commonly known acronyms may be used, and they should be spelt out at first use followed by the abbreviation in brackets. SI units should be used, with litre and molar being permitted.
• **Units of measurement:** Measurements should be given in SI or SI-derived units. Visit the Bureau International des Poids et Mesures (BIPM) website here for more information about SI units.
• **Numbers:** Numbers under 10 are spelt out, except for: measurements with a unit (8mmol/l); age (6 weeks old), or lists with other numbers (11 dogs, 9 cats, 4 gerbils).
• **Trade Names:** Chemical substances should be referred to by the generic name only. Trade names should not be used. Drugs should be referred to by their generic names. If proprietary drugs have been used in the study, refer to these by their generic name, mentioning the proprietary name and the name and location of the manufacturer in parentheses.
• **Footnotes:** Footnotes are not allowed in the text, the information shall be included directly into the text, where it fits best, and if these are references, to include in the reference section at the end.
• **Language**: All submissions must be in UK English (International) and UN-accepted terminology should be followed. No capitalization should be used except for grammatically correct use, official names and titles, and abbreviations.
• **General recommendation:** Use line spacing of 1.5 and an easily readable font, for example, Times New Roman, size 12. Your manuscript should contain line numbers to facilitate editors' and reviewers' comments

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